

CHRONIC TOXICITY SUMMARY

# ISOPHORONE

(1,1,3-trimethyl-3-cyclohexene-5-one; 3,5,5-trimethyl-2-cyclohexene-1-one; isoforon;  
isoacetophorone)

CAS Registry Number: 78-59-1

## I. Chronic Toxicity Summary

<i>Inhalation reference exposure level</i>	<b>2,000 µg/m<sup>3</sup></b>
<i>Critical effect(s)</i>	Developmental effects (reduced crown-rump length of female rat fetuses) and teratogenicity (exencephaly) in fetal rats and mice
<i>Hazard index target(s)</i>	Development

## II. Chemical Property Summary (HSDB, 1995)

<i>Molecular formula</i>	C <sub>9</sub> H <sub>14</sub> O
<i>Molecular Weight</i>	138.21
<i>Description</i>	Water-clear liquid with a peppermint-like odor
<i>Vapor Pressure</i>	0.44 mm Hg at 25°C
<i>Solubility</i>	Slightly soluble in water, 12,000 mg/l water at 25°C. Miscible in organic solvents.
<i>Conversion factor</i>	5.65 µg/m <sup>3</sup> per ppb at 25°C

## III. Major Uses and Sources

Isophorone is used extensively as a solvent in some printing inks, paints, lacquers, adhesives, vinyl resins, copolymers, coatings, finishes, and pesticides, in addition to being used as a chemical intermediate (HSDB, 1995). Since this compound has many different applications, release to the environment may originate from a wide variety of industrial sources including iron and steel manufacturers, manufacturers of photographic equipment and supplies, automobile tire plants and printing operations. Coal-fired power plants may also emit isophorone to the air. Although it is mostly a man-made compound, isophorone has been found to occur naturally in cranberries (ATSDR, 1989). Due to its high water solubility and short half-life in the atmosphere ( $t_{1/2} < 5$  hrs), the most probable route of exposure to isophorone for the general population is ingestion of contaminated drinking water. Individuals living near hazardous waste sites may also be exposed to isophorone dermally, but not by inhalation (ATSDR, 1989). Occupational exposure may occur by inhalation or dermal contact.

#### IV. Effects of Human Exposures

In occupational monitoring studies, the time-weighted average concentration in breathing zones and workplace air of a screening plant ranged from 8.3-23 ppm and from 3.5-14.5 ppm, respectively (Samimi, 1982). Up to 25.7 ppm was detected in air of a silk screening printing plant in Pittsburgh, PA (Kominsky, 1983). The concentration in breathing zone samples from a decal manufacturing plant in Ridgefield, NJ was 0.7-14 ppm (Lee and Frederick, 1982). It was suspected that the reported eye and nose irritation of workers at the silk screening plant and at the decal manufacturing plant were the result of acute and subacute exposure to isophorone vapors.

No information is available concerning long-term exposure or pharmacokinetics of isophorone in humans (ATSDR, 1989). However, workers exposed to 5-8 ppm (28-45 mg/m<sup>3</sup>) of isophorone for one month complained of fatigue and malaise (NIOSH, 1978). When concentrations were reduced to 1-4 ppm, no adverse effects were reported. Acute exposure studies in humans (up to 400 ppm for 1 to 4 minutes) resulted in eye, nose and throat irritation, nausea, headache, and dizziness or faintness (Union Carbide, 1963). Fifteen minute inhalation exposure to 10 ppm isophorone produced only mild effects in human subjects while 25 ppm produced irritation to eyes, nose, and throat (Silverman *et al.*, 1946).

#### V. Effects of Animal Exposures

Few reports have been published regarding the pharmacokinetics of isophorone in experimental animals. Isophorone was widely distributed in the major organs of the rat following 4 hour inhalation exposure to 400 ppm (ATSDR, 1989). Oral gavage of 4000 mg/kg body wt to rats and a rabbit also resulted in wide distribution of the chemical. The highest blood levels of isophorone were reached by 30 min in rabbits following oral gavage and had decreased dramatically by 21 hours, indicating rapid absorption and elimination of the chemical. Preliminary results of a pharmacokinetic study indicate that rats treated orally with <sup>14</sup>C-isophorone excreted 93% of the radiolabel in the urine, expired air, and feces in 24 hours (ATSDR, 1989). The highest levels of <sup>14</sup>C-isophorone were found in the liver, kidney, preputial gland, testes, brain, and lungs. Several metabolites were identified in the urine of orally dosed rats and rabbits, including 3-carboxy-5,5-dimethyl-2-cyclohexene-1-one, 3,5,5-trimethylcyclohexanol, and some glucuronide conjugates (Dutertre-Catella *et al.*, 1978). A portion of the chemical was excreted unchanged in expired air.

In an early inhalation study, 10 Wistar rats/group and 10 guinea pigs/group, all of mixed sex, were exposed to 0, 25, 50, 100, 200 or 500 ppm isophorone 8 hr/day, 5 days/week for 6 weeks (Smyth *et al.*, 1942). Increased mortality and reduced body weights were observed at 100 ppm and up in both species. However, eye and nose irritation was noted only at the highest dose. Minor changes in blood chemistry (increased polymorphonuclear cells and decreased lymphocytes) and urinalysis (increased albumin) of guinea pigs were seen only at the highest dose. Histopathology of the livers revealed no convincing treatment-related effect. Dilation of

Bowman's capsule and cloudy swelling of the convoluted tubular epithelium occurred in kidneys of animals (assumed to be both species) at 50 ppm and up. However, two controls also had slight lesions of the tubular epithelium. It was reported that lungs were often congested but dose levels for corresponding lung lesions were not provided. Later investigations determined that the isophorone used in this study was contaminated with appreciable amounts of compounds more volatile than isophorone (Rowe and Wolf, 1963). Therefore, some of the adverse effects (i.e. the lung lesions) may have been due to the contaminants. The presence of highly volatile contaminants would also result in inaccurate concentrations of isophorone used in the study.

In a 90-day feeding study, 20 CFE albino rats/group/sex and 4 beagle dogs/group/sex were given isophorone in their diet (rats) at concentrations of 0, 750, 1500 or 3000 ppm, or in gelatin capsules (dogs) at concentrations of 0, 35, 75 or 150 mg/kg body wt-day (AME, 1972a,b). High dose rats exhibited slightly reduced weight gain compared to controls (8-10%) for most of the study. Average weight gain among the exposure groups of beagle dogs remained essentially unchanged during the entire study. Urinalysis, hematology and clinical chemistry indices found no treatment-related effects in the animals at either the interim or final toxicological examinations. Gross pathology and a limited histopathological examination observed no treatment-related effects in either species. Data on isophorone purity and possible loss of isophorone from rat diet due to vaporization were not presented.

In the most comprehensive isophorone toxicity study to date, 50 F344/N rats/group/sex and 50 B6C3F1 mice/group/sex were administered 0, 250 or 500 mg isophorone/kg body wt 5 days/week by oral gavage (in corn oil) for 103 weeks (Bucher *et al.*, 1986; NTP, 1986). Clinical signs of toxicity were not seen during the length of the study. However, several deaths in male and female rats at the high dose occurred early in the study. A steep decline in survival rate of high dose male rats occurred after week 90. Male and female rats and female mice in the high dose group exhibited only a slight decrease in body weight (<10%) compared to controls. A 13-week range finding study for the 2-year study did not find compound-related lesions in the kidney (or any other organs) of rats and mice exposed up to 1000 mg/kg body wt-day. However, pathological examination of rats exposed to isophorone for 2 years revealed non-neoplastic lesions in the kidney. Increased mineralization of the collecting ducts in isophorone-exposed male, but not female, rats was observed. This lesion was characterized by basophilic aggregates of mineral most often found in the medullary collecting ducts and occurred coincidentally with lesions of chronic nephropathy. Nephropathy was observed in almost half the female controls and nearly all the male controls. Isophorone exposure appeared to increase both the severity of nephropathy in low dose male rats and the incidence of nephropathy in dosed female rats, but the effects were not pronounced. However, the isophorone potentiation of nephropathy in rats may be due to 'male rat-specific nephropathy' and not have any relevance to human exposure (Strasser *et al.* 1988). Other adverse effects in kidneys of isophorone-treated male rats include tubular cell hyperplasia (in a dose-related manner) and epithelial hyperplasia of the renal pelvis. In mice, an increased incidence of chronic focal inflammation was observed in the kidneys of males, but was not considered treatment-related. A dose-dependent increase in fatty metamorphosis occurred in the adrenal cortex of male rats, but the biological significance of this change is unknown. All isophorone-exposed male mice had an increased incidence of hepatocytomegaly and coagulative necrosis of the liver. However, treatment-related liver lesions

were not observed in female mice. Increased incidence of hyperkeratosis of the forestomach was observed in dosed male and high dose female mice, but was probably not a treatment-related effect.

Published studies on possible reproductive effects of isophorone are lacking. An unpublished inhalation study conducted by a commercial laboratory (Bio/dynamics, 1984b) studied possible teratogenicity due to isophorone in rats or mice at inhaled doses up to 115 ppm. Groups of 22 female rats and 22 female mice were exposed to 0, 25, 50 or 115 ppm isophorone (6 hr/day) on gestational days 6-15. Maternal toxicity in rats included dose-dependent alopecia and cervical/anogenital staining. Low body weights (7-8%) were occasionally observed in the 115 ppm group. In mice, maternal toxicity was confined to slightly decreased weight (7-8%) on one day in the 115 ppm group. No significant differences were found in uterine implantations, fetal toxicity, and external and internal malformations among the animals. However, a slight, but significant, growth retardation in the form of decreased crown-rump length was present among the high dose fetal rats. Also, a slight, but insignificant, increase in extra ribs and/or rudimentary ribs was seen in rat and mouse fetuses at the highest dose. In a pilot study for this developmental toxicity investigation (12 females/species), exencephaly was observed in 1 rat and 1 mouse undergoing late reabsorption and in 2 live rat fetuses from dams exposed to 150 ppm isophorone on gestational days 6-15 (Bio/dynamics, 1984a). Exencephaly was not observed at any dose level in the primary study.

Contrary to the findings of the above report, Dutertre-Catella (1976) did not find adverse reproductive or developmental effects in rats exposed to 500 ppm isophorone (6 hr/day, 5 days/week) for 3 months before mating. Females had been exposed throughout gestation as well. The pups were not examined for internal malformations so the study was incomplete for determination of developmental effects.

## VI. Derivation of Chronic Reference Exposure Level (REL)

<i>Study</i>	Bio/dynamics 1984a,b
<i>Study population</i>	22 female mice/group, 22 female rats/group
<i>Exposure method</i>	Discontinuous whole body inhalation exposure during gestation (0, 25, 50 or 115 ppm)
<i>Critical effects</i>	Developmental effects (reduced crown-rump length of female rat fetuses) and teratogenicity (exencephaly in fetal rats and mice)
<i>LOAEL</i>	115 ppm
<i>NOAEL</i>	50 ppm
<i>Exposure continuity</i>	6 hr/day during gestation
<i>Exposure duration</i>	Days 6-15 of gestation
<i>Average experimental exposure</i>	12.5 ppm (50 x 6/24)
<i>Human equivalent concentration</i>	12.5 ppm (gas with systemic effects, based on RGDR = 1.0 using default assumption that $\lambda(a) = \lambda(h)$ )

<i>LOAEL uncertainty factor</i>	1
<i>Subchronic uncertainty factor</i>	1
<i>Interspecies uncertainty factor</i>	3
<i>Intraspecies uncertainty factor</i>	10
<i>Cumulative uncertainty factor</i>	30
<i>Inhalation reference exposure level</i>	0.4 ppm (400 ppb, 2 mg/m <sup>3</sup> , 2,000 µg/m <sup>3</sup> )

The inhalation study by Bio/dynamics (1984a,b) presents data that indicate exposure during gestation may be the most sensitive indicator of non-neoplastic toxicity by isophorone. Exposure of pregnant rats to 115 ppm isophorone during gestation resulted in significant growth retardation of female rat fetuses (reduced crown-rump length). Exposure to 50 ppm isophorone, the NOAEL, produced no developmental effects. The authors claim that removal of the two shortest female fetuses from the statistical analysis results in no significant difference for growth retardation; therefore, this adverse effect is not significant. However, this selective culling before the statistical analysis is not scientifically appropriate in this case. In addition, the authors did not perform some of the scheduled fetal examinations. Otherwise, the growth retardation might have had even greater statistical significance. The pilot study (Bio/dynamics, 1984a) observed exencephaly in a few mouse and rat fetuses at 150 ppm. Exencephaly was not considered significant by the authors because it was not present in any fetuses of the primary study (Bio/dynamics, 1984b). However, exencephaly is included as a critical effect in this summary because it is considered a serious teratogenic effect that was present at a dose only slightly higher than the LOAEL of the primary study (115 ppm). Alopecia of adult female rats was observed in many of the exposed animals. However, this effect may be considered more of an acute dermal irritation than a chronic effect. In addition, cervical and anogenital staining seen in many exposed rats is not considered an 'adverse' effect.

A strength of the database for isophorone is the consistency of the REL when compared to the study by Bucher *et al.* (1986). Developing a chronic REL based on the adverse effects due to lifetime exposure (Bucher *et al.*, 1986) results in about the same REL (0.2 ppm) as that produced due to adverse effects during gestation (Bio/dynamics, 1984a,b). Weaknesses of the database for isophorone include the lack of human exposure data and the lack of long-term inhalation studies. However, the lack of human data may be due to isophorone's rather low potency for causing chronic, non-neoplastic, adverse effects. Also, inhalation of isophorone is most likely a minor route of exposure for the general population. Due to the insufficient characterization of the kidney nephropathy in the NTP study (Bucher *et al.*, 1986; NTP, 1986), a subchronic or chronic study in a non-rodent species would enhance the database for isophorone. Another comprehensive study of possible reproductive and developmental effects of isophorone in experimental animals would also strengthen the database.

## VII. References

AME. 1972a. Affiliated Medical Enterprises, Inc. 90-Day subchronic toxicity of isophorone in the rat (final report). Unpublished study performed by Affiliated Medical Enterprises, Inc.

Princeton, N.J. for Rohm and Haas Co. Philadelphia, PA. OTS 8d submission Doc. ID. 87812179, Microfiche No. 205975.

AME. 1972b. Affiliated Medical Enterprises, Inc. 90-Day subchronic toxicity of isophorone in the dog (final report). Unpublished study performed by Affiliated Medical Enterprises, Inc. Princeton, N.J. for Rohm and Haas Co. Philadelphia, PA. OTS 8d submission Doc. ID. 87812178, Microfiche No. 205975.

ATSDR. 1989. Agency for Toxic Substances and Disease Registry. Toxicological profile for isophorone. U.S. Public Health Service, Atlanta, GA. PB90-180225.

Bio/dynamics. 1984a. Inhalation teratology probe study in rats and mice. Project No. 323771. Unpublished study performed by Bio/dynamics Inc. East Millstone, NJ. OTS Section 4 submission Doc. ID 40-8455042. Microfiche No. OTS0507219, pp. 1-33.

Bio/dynamics. 1984b. Inhalation teratology study in rats and mice. Final Report 3223772. Unpublished study performed by Bio/dynamics Inc. East Millstone, NJ for Exxon Biomedical Science, East Millstone NJ. OTS Section 4 submission Doc. ID 40-855049. Microfiche No. OTS 0507224, pp. 1-107.

Bucher Jr, Huff J, and Kluwe WM. 1986. Toxicological and carcinogenesis studies of isophorone in F344 rats and B6C3F1 mice. Toxicology 39:207-219.

Dutertre-Catella H. 1976. Contribution to the analytical toxicological and bio-chemical study of isophorone (in French). Thesis for doctorate in pharmacology, Universite Rene Descartes, Paris. [Cited in Joint Assessment of Commodity Chemicals, No. 110, Isophorone, ECETOC, Brussels, 1989.]

Dutertre-Catella H, Nguyen PL, Dang Quoc Q, and Truhaut R. 1978. Metabolic transformations of the 3,5,5-2-cyclohexene-1-one trimethyl (isophorone). Toxicol. Eur. Res., 1(4):209-216.

HSDB. 1995. Hazardous Substances Data Bank. National Library of Medicine, Bethesda, MD (CD-ROM Version). Micromedex, Inc., Denver, CO (Edition expires 11/31/95).

Kominsky JR. 1981. Health hazard determination report no. HE 78-107-563, Swinston Company, Pittsburgh, PA.

Lee SA, and Frederick L. 1982. NIOSH health hazard evaluation report no. HHE80-103-827; NTIS PB82-189226.

NIOSH. 1978. National Institute for Occupational Safety and Health. Occupational exposure to ketones: criteria for a recommended standard. U.S. Department of Health, Education, and Welfare. DHEW (NIOSH) Publication No. 78-173.

NTP. 1986. National Toxicology Program. Toxicology and carcinogenesis studies of isophorone in F/344 rats and B6C3F<sub>1</sub> mice. NTP TR 291, NIH Publication No. 86-2547.

Rowe VK, and Wolf MA. 1963. Ketones. In: Industrial Hygiene and Toxicology, Second ed. Patty, F. A. (ed) Interscience Publishers, New York, p.1764.

Samimi B. 1982. Exposure to isophorone and other organic solvents in a screen printing plant. Am. Indus. Hyg. Assoc. J., 43(1):43-48.

Silverman L, Schulte HF, and First MW. 1946. Further studies on sensory response to certain industrial solvent vapors. J. Indus. Hyg. Toxicol., 28(6):262-266.

Strasser J Jr, Charbonneau M, Borgoff SJ, Turner MJ, and Swenberg JA. 1988. Renal protein droplet formation in male Fischer 344 rats after isophorone (IPH) treatment. Toxicologist, 8(1):136.

Smyth HF Jr, Seaton J, and Fischer L. 1942. Response of guinea pigs and rats to repeated inhalation of vapors of mesityl oxide and isophorone. J. Indus. Hyg. Toxicol., 24(3):46-50.

Union Carbide Corporation. 1963. Toxicology Studies--Isophorone Summary Data Sheet. New York: Ind. Med. Toxicol. Dept.

CHRONIC TOXICITY SUMMARY

# ISOPROPANOL

(2-propanol; dimethylcarbinol; isopropyl alcohol)

CAS Registry Number: 67-63-0

## I. Chronic Toxicity Summary

<i>Inhalation reference exposure level</i>	2,000 $\mu\text{g}/\text{m}^3$
<i>Critical effect(s)</i>	Increased liver weights in mice
<i>Hazard index target(s)</i>	Alimentary

## II. Chemical Property Summary (HSDB, 1995)

<i>Molecular formula</i>	$\text{C}_3\text{H}_8\text{O}$
<i>Molecular Weight</i>	60.09
<i>Description</i>	Colorless liquid at room temperature (25°C) with a pleasant odor. Slightly bitter taste.
<i>Vapor Pressure</i>	44.0 mm Hg at 25°C
<i>Solubility</i>	Miscible in water and most organic solvents. Insoluble in salt solutions.
<i>Conversion factor</i>	1 ppb = 2.45 $\mu\text{g}/\text{m}^3$ at 25°C

## III. Major Uses and Sources

Isopropyl alcohol is used as a solvent and in making many commercial products (HSDB, 1995). The annual production volume of isopropyl alcohol has been in excess of one billion pounds since 1956; it was ranked 50<sup>th</sup> among chemicals produced in the U.S. in 1994 (C&EN, 1995). Rubbing alcohol is a solution of 70% isopropyl alcohol in water. Specific uses and sources include component of antifreeze; solvent for gums, shellac, essential oils, creosote and resins; extraction of alkaloids; component of quick drying oils and inks; component of denaturing alcohol; antiseptic for hand lotions; rubefacient; component of household products (after-shave lotions, cosmetics, etc.); the manufacture of acetone; deicing agent for liquid fuels; dehydrating agent and synthetic flavoring adjuvant. Isopropyl alcohol can enter the environment as emissions from its manufacture and use as a solvent. It naturally occurs as a plant volatile and is released during the microbial degradation of animal wastes. Human exposure will be both in occupational atmospheres and from use of consumer products containing isopropyl alcohol as a volatile solvent. An odor threshold has been estimated as 22 ppm (Amoore and Hautala, 1983), which is 10-fold higher than the chronic REL.

#### **IV. Effects of Human Exposures**

Currently, there is no adequate chronic exposure data in humans. However, many other solvents and petroleum-based chemicals have been shown to cause brain or other nerve damage with prolonged exposure. While isopropyl alcohol is not considered a dermal irritant, it is a defatting agent and can cause dermatitis with prolonged exposure to skin (IARC, 1977). A subacute study of daily oral intake of isopropyl alcohol (2.6 or 6.4 mg/kg body weight) by groups of 8 men for 6 weeks had no effect on blood cells, serum or urine and produced no subjective symptoms (Wills *et al.*, 1969). A pharmacokinetic study of men occupationally exposed to isopropyl alcohol revealed uptake occurs readily via the inhalation route with acetone as the major metabolite (Brugnone *et al.*, 1983). Acetone was eliminated mainly by the lung but was also eliminated in the urine.

#### **V. Effects of Animal Exposures**

In metabolism studies with rats and mice, up to 92% of the administered dose (via i.v. or inhalation) of isopropyl alcohol was exhaled as acetone, CO<sub>2</sub> and the unmetabolized alcohol (Slauter *et al.*, 1994). Approximately 3-8% of the administered dose was excreted in urine as isopropyl alcohol, acetone, and a metabolite tentatively identified as isopropyl glucuronic acid. Isopropyl alcohol is readily absorbed from the GI tract and persists in the circulation longer than ethyl alcohol. Alcohol dehydrogenase oxidizes most isopropyl alcohol to acetone. Acetone may be further metabolized to acetate, formate, and finally CO<sub>2</sub>. In another metabolism study, the amount of acetone in the blood stream was found to be directly related to the air concentration of isopropyl alcohol (Laham *et al.*, 1980). This finding indicated that the acetone metabolite could be used as a biochemical indicator of isopropyl alcohol exposure.

No long-term studies, spanning a majority of the lifespan of the test animal, have been performed with isopropyl alcohol. However, several subchronic inhalation studies exist in the literature. Subchronic studies by Guseinov and Abasov (1982) and Baikov *et al.* (1974) reported changes in certain hematologic and clinical chemistry parameters, as well as increases in some organ weights. But the Environmental Protection Agency deemed these studies insufficient to reasonably predict subchronic toxicity of isopropyl alcohol (Burleigh-Flayer *et al.*, 1994). Three different routes of exposure have been used by researchers for isopropyl alcohol toxicity studies: inhalation, oral gavage and presence in drinking water. The following 5 studies exposed experimental animals to isopropyl alcohol by the inhalation route:

The most comprehensive recent report investigated subchronic and neurobehavioral endpoints in rats and mice following 13-week inhalation exposure (6 hr/day, 5 days/week) to 0, 100, 500, 1500 or 5000 ppm isopropyl alcohol (Burleigh-Flayer *et al.*, 1994). In rats, clinical signs observed following exposures included swollen periocular tissue (females) at the highest dose and perinasal encrustation (males) at 500 ppm and above. Narcosis was observed in a few animals of both species during exposure to 5000 ppm and possibly 1500 ppm as well. However, the animals became tolerant to the narcotic effects of isopropyl alcohol after week 2. No neurobehavioral changes were observed in any parameters of the functional observational battery.

However, increased motor activity was noted at week 9 of exposure in female rats of the 5000 ppm group. After an initial drop in body weight gain in the first week of exposure at the high dose (5000 ppm), rats in the 1500 and 5000 ppm groups had significant increases in body weight gain and/or body weight throughout most of the exposure period. But only the 5000 ppm group had greater than 10% body weight gain compared to controls. Increases in body weight and body weight gain greater than 10% were also noted in female mice in the 5000 ppm group. Consistent clinical pathology changes included an increase in mean corpuscular volume (rats; female mice) and mean corpuscular hemoglobin (male rats; female mice) at the 5000 ppm exposure level. Other changes noted include a slight anemia in rats at week 6 only and a slight dehydration in female mice at the end of the study. Relative liver weight in rats was elevated no more than 8% in the 5000 ppm groups. However, a 10 and 21% increase in relative liver weight was observed in female mice at 1500 and 5000 ppm, respectively. No gross lesions were observed in any organs and the only microscopic change observed was hyaline droplets within kidneys of all male rats. This change was not clearly concentration related, although this microscopic change was most pronounced in the 5000 ppm group. The hyaline droplets found in kidneys of male rats has been shown to be a male rat-specific phenomenon and is not considered to be relevant to human risk assessment (Phillips and Cockrell, 1984; Beyer, 1992).

In a similar 13-week behavioral/neurotoxicity study by the same investigators, increased motor activity in female Fischer 344 rats was also seen during exposure to 5000 ppm (12,300 mg/m<sup>3</sup>) isopropyl alcohol (Union Carbide Corp., 1990). Increased motor activity was characterized as the summation of ambulation, rearing and fine movements. Complete recovery was apparent within 42 days post-exposure. Other effects included a significant increase in body weight and an increased incidence of swollen periocular tissue in isopropyl alcohol-exposed animals.

In a study conducted to investigate neurochemical and behavioural effects, 20 male Wistar rats/group were exposed to 0 or 300 ppm isopropyl alcohol 6 hr/day, 5 days/week for up to 21 weeks (Savolainen *et al.*, 1979). Enzyme activity of superoxide dismutase and azoreductase in cerebellar homogenate was decreased at week 20-21. Acid protease activity in glial cells was increased up to week 10. Open-field tests indicated sporadic changes in urination (10th week) and defecation (15th week). Isopropyl alcohol also appeared to depress caffeine stimulation activity at 15 weeks.

In a subchronic neurotoxicity study by Teramoto *et al.* (1993), motor and sensory nerve conduction velocity increased significantly following a 20-week exposure (8 hr/day, 5 days/week) of Jcl-Wistar rats to 8000 ppm isopropyl alcohol. Low dose (1000 ppm) exposure had no effect on conduction velocity. Conduction velocities returned to normal following the end of exposure. The sex of the rats in this study was not specified.

A developmental study in rats exposed pregnant dams (15/group) to 0, 3500, 7000 or 10,000 ppm isopropyl alcohol 7 hr/day on gestation days 1-19 (Nelson *et al.*, 1988). At the highest exposure level, maternal body-weight gain and food consumption were reduced. Narcosis was also evident. At 7000 ppm isopropyl alcohol, only body-weight gain was slightly reduced. Increased fetal resorptions and reduced fetal weights (41%) occurred at the highest exposure level. Fetal weights were also significantly reduced at 7000 ppm (15%) and at 3500 ppm (4%), showing a

dose-dependent relationship. Skeletal malformations were seen only in the presence of maternal toxicity at the two highest exposure levels. No detectable teratogenic effects were observed in the 3500 ppm group.

The following toxicology studies administered isopropyl alcohol to experimental animals by oral gavage:

In a developmental study, pregnant (VAF)CD(SD) rats (25/group) were gavaged with either 0, 400, 800 or 1200 mg/kg body wt-day of isopropyl alcohol daily on gestational days 6 through 15 (Tyl *et al.*, 1994). In the same study, pregnant New Zealand white rabbits (15/group) were dosed orally with either 0, 120, 240 or 480 mg/kg body wt-day of isopropyl alcohol daily during gestational days 6 through 18. In rats, fetal body weights exhibited a linear downward trend with increasing dose and was significantly lower at the highest dose compared to controls. However, the fetal body weight differences at each dose level was less than 10% of controls. Maternal weight gain during gestation was significantly reduced at the highest dose level. In rabbits, maternal weight gain and food consumption were reduced during gestation at 480 mg/kg body wt-day. Four rabbits died after dosing at this level. No differences were observed in reproduction indices or in fetal development. No teratogenic effects were seen in either species.

In another developmental study performed to investigate neurotoxicity in rat pups, 64 time-mated Sprague-Dawley rats/group were administered 0, 200, 700 or 1200 mg/kg body wt-day isopropyl alcohol by oral gavage from gestational day 6 through postnatal day 21 (Bates *et al.*, 1994). One high-dose dam died on postnatal day 15, but there were no other clinical observations of effects on maternal weight, food consumption or gestation length. All fetal developmental indices were unaffected at the dose levels used. Developmental neurotoxicity, in the form of motor activity, auditory startle and active avoidance tests, was not found at any dose of isopropyl alcohol.

In a multi-generation study carried out to investigate potential reproductive and developmental effects of isopropyl alcohol, Sprague-Dawley rats were administered 0, 100, 500 or 1000 mg/kg body wt-day of isopropyl alcohol by oral gavage (Beyer, 1992). These doses are approximately equal to 0, 140, 710 and 1400 ppm isopropyl alcohol, respectively. P1 and P2 rats were dosed daily for 10 weeks prior to mating and throughout the mating, gestation and lactation period for the F1 and F2 litters, respectively. In adult rats, centrilobular hepatocyte hypertrophy and increased relative liver weight (>10%) was observed in P2 males at 1000 mg/kg. A general increase in absolute and relative liver and kidney weights were observed (less than 10% in most cases) in treated animals in both P1 and P2 generations. However, with the exception of hepatocellular hypertrophy in P2 males, no histopathological effects relevant to human risk were present. Reproductive effects due to isopropyl alcohol were not seen at any dose level. Statistically significantly reduced body weights (5-12%) in F1 and F2 offspring and increased mortality (14%) in F1 offspring were observed at the 1000 mg/kg dose level.

The following toxicology studies administered isopropyl alcohol to experimental animals in drinking water:

In a study designed to investigate neurotoxicological effects, 22 male SPF rats/group were administered isopropyl alcohol in drinking water at concentrations of 0, 1, 2, 3, 4 or 5% (w/v) for 12 weeks (Pilegaard and Ladefoged, 1993). Average daily intake was 0, 870, 1280, 1680 and 2520 mg/kg body wt., respectively. Water intake and body weights were consistently lower at the two highest doses. Relative weights of liver, kidney and adrenals were increased in a dose-dependent manner. However, histopathology revealed no treatment-related changes in organs other than the male rat-specific kidney lesions. Evidence of astrogliosis, in the form of increased glial fibrillary acidic protein in dorsal hippocampus, was not found in exposed rats.

In a 1-generation study, 10 Wistar-derived rats/sex/group were exposed to 0, 0.5, 1.25, 2.0, and 2.5% isopropyl alcohol (equivalent to 0, 325, 711, 1002 and 1176 mg/kg body wt-day, respectively, for males; 517, 1131, 1330 and 1335 mg/kg body wt-day, respectively, for females during pre-mating phase; and, 1167, 2561, 2825 and 2722 mg/kg body wt-day, respectively, in females during the post-partum phase) in water for up to 18 weeks (USEPA/OTS, 1986). Exposure periods were: 70 days pre-mating, plus 15 days during mating, plus 42 days for males; 21 days pre-mating plus 15 days during mating, plus 21 days gestation, plus 21 days rearing in females; and 21 days for the F<sub>1</sub> generation. At the highest two levels, body weights of males during the first two weeks were reduced and the body weights of females during the post-partum period were reduced. Water consumption and food ingestion were generally lower at the top three dose levels. The authors concluded that these effects were related to the unpalatability of drinking water containing isopropyl alcohol and not due to a toxic effect of the alcohol itself. Anemia was present in post-partum females. Red cell numbers were reduced in a dose-related manner at doses of 1.25% isopropyl alcohol or higher. Hematocrit was lower at the two highest doses while hemoglobin was lower at the highest dose. In males, mean cell volume was reduced at 1.25% isopropyl alcohol or higher. Absolute and relative liver and kidney weights were higher in most exposure groups at 2.0% or higher in both sexes, but no relevant pathology was seen. Absolute liver weight of females was also higher in the 1.25% group. Fetal weight gain was depressed in a dose-related fashion in the 1.25% and higher groups. Mean pup weights and pup survival were lower than controls at the two highest doses. Fewer pups were born per animal in the 2.5% exposure group. A teratogenic examination was not performed on the pups.

In a similar exposure study investigating the potential teratogenic effects of isopropyl alcohol, 20 pregnant Wistar-derived rats/group were exposed to 0, 0.5, 1.25 or 2.5% of the alcohol in drinking water (equivalent to 0, 596, 1242 and 1605 mg/kg body wt-day) during gestational days 6 to 16 (USEPA/OTS, 1992a,b). Water and feed consumption were reduced at the two highest doses while maternal body weight was significantly reduced at the highest dose. Fetal body weights were decreased in the 1.25 and 2.5% groups. Minor abnormalities and variants (reduced ossification of the skeleton) were present in fetuses of exposed groups in a dose-related manner. However, the authors concluded that the reduced fetal weights are probably a consequence of maternal growth retardation during the critical period of organogenesis. Similarly, the fetal abnormalities are probably due to small fetal size, related to slightly retarded development. Therefore, the study found no indication of teratogenesis.

A multi-generation study performed in 'white' rats also observed reduced body weights in F<sub>1</sub> offspring (Lehman *et al.*, 1945). Body weights of F<sub>2</sub> offspring were the same as controls. The

adult rats had imbibed an average of 1.9 cc/kg (1470 mg/kg body wt) of isopropyl alcohol per day in drinking water 80 days prior to mating. No other developmental or reproductive effects were seen. In the same study, several dogs were given 4% isopropyl alcohol in drinking water for approximately 7 months. Histopathology at the end of exposure revealed a decrease in the number of nephrons with hydropic changes and necrosis of some of the tubular epithelium. Some capillary hemorrhages were also noted in the brains of two of the dogs. Average daily dose of isopropyl alcohol imbibed by the dogs could not be determined from data provided in the report.

## VI. Derivation of Chronic Reference Exposure Level (REL)

<i>Study</i>	Burleigh-Flayer <i>et al.</i> 1994)
<i>Study population</i>	Rats and mice
<i>Exposure method</i>	Discontinuous whole-body inhalation (0, 100, 500, 1500 or 5000 ppm)
<i>Critical effects</i>	Increased relative liver weight (10% over controls) (female mice); hyaline droplets in kidneys (male rats only)
<i>LOAEL</i>	1,500 ppm
<i>NOAEL</i>	500 ppm
<i>Exposure continuity</i>	6 hours/day, 5 days/week
<i>Exposure duration</i>	13 weeks
<i>Average experimental exposure</i>	89 ppm for NOAEL group (500 x 6/24 x 5/7)
<i>Human equivalent concentration</i>	89 ppm for NOAEL group (gas with systemic effects, based on RGDR = 1.0 using default assumption that $\lambda(a) = \lambda(h)$ )
<i>LOAEL uncertainty factor</i>	1
<i>Subchronic uncertainty factor</i>	3
<i>Interspecies uncertainty factor</i>	3
<i>Intraspecies factor</i>	10
<i>Cumulative uncertainty factor</i>	100
<i>Inhalation reference exposure level</i>	0.9 ppm (900 ppb, 2 mg/m <sup>3</sup> , 2,000 µg/m <sup>3</sup> )

Many studies also noted increased liver and kidney weights in exposed animals but with no observable relevant pathology. This change may be considered more of a metabolic response, rather than a toxic effect, of the alcohol. Several studies noted hyaline droplets and other lesions in kidneys characteristic of a male rat-specific phenomenon, but it is not relevant to human toxicity. The changes noted in the neurochemical and behavioural study by Savolainen *et al.* (1979) may have also been more of a metabolic response to the increased load of isopropyl alcohol. Its also possible that these changes reflected a development of tolerance. The changes in behavior were small and unconvincing. This study would have benefited from additional dose levels to analyze for dose-response trends.

Sensitive indicators of chronic adverse effects from isopropyl alcohol exposure include development of tolerance to narcosis, blood chemistry changes and reduced fetal body weights. Burleigh-Flayer *et al.* (1994) noted a development of tolerance in rats to the narcotic effects of isopropyl alcohol at 5000 ppm. Burleigh-Flayer *et al.* (1994) and USEPA/OTS (1986) observed blood changes characteristic of anemia in rats at 5000 ppm and 2561 mg/kg body wt-day (equiv. to 3660 ppm), respectively. The most sensitive indicator of chronic oral isopropyl alcohol exposure appears to be the reduction of fetal body weights in developmental studies. This effect occurred in studies using exposure routes via inhalation, drinking water and oral gavage. In addition, reduced fetal body weights occurred at doses lower than the observed reduction of maternal body weights. In studies by Nelson *et al.* (1988), Tyl *et al.* (1994), USEPA/OTS (1986), USEPA/OTS (1992a,b) and Beyer (1992), LOAELs for significantly reduced fetal body weights occurred at 3500 ppm, 400 mg/kg body wt-day (equiv. to 570 ppm), 2560 mg/kg body wt-day (equiv. to 3660 ppm), 1240 mg/kg body-wt day (equiv. to 1770 ppm) and 1000 mg/kg body wt-day (equiv. to 1430 ppm), respectively. The study by Tyl *et al.* (1994) resulted in the smallest LOAEL (570 ppm) for chronic adverse effects to isopropyl alcohol. It should be noted that skeletal malformations, probably related to reduced fetal weight, were observed in 2 studies (Nelson *et al.*, 1988; USEPA/OTS, 1992a,b).

Strengths of the database for isopropyl alcohol, besides similar toxicological endpoints among different studies, include pharmacokinetic similarities between humans and experimental animals. These studies show that isopropyl alcohol is metabolized through a similar pathway to acetone and CO<sub>2</sub>.

Weaknesses of the database for isopropyl alcohol include the lack of lifetime exposure studies in experimental animals and a lack of literature regarding chronic toxicity endpoints in humans. The deficiency of chronic toxicity cases in humans may be related to the relatively low chronic toxicity of isopropyl alcohol. A thorough chronic or subchronic toxicity study in a non-rodent species would also enhance the database. Another weakness is that while most developmental studies observed maternal and fetal effects, one recent study found no such effects at equivalent doses (Bates *et al.*, 1994).

## VII. References

Amoore JE, and Hautala E. Odor as an aid to chemical safety: Odor thresholds compared with threshold limit values and volatiles for 2114 industrial chemicals in air and water dilution. *J Appl Toxicol* 1983;3(6):272-290.

Baikov BK, Gorlova OE, Gusev MI, Novikov Yu V, Yudina TV, and Sergeev AN. 1974. Hygienic standardization of the daily average maximum admissible concentrations of propyl and isopropyl alcohols in the atmosphere. *Gigiena i Sanitariia*, 4:6-13.

Bates HK, McKee RH, Bieler GS, Gardiner TH, Gill MW, Strother DE, and Masten LW. 1994. Developmental neurotoxicity evaluation of orally administered isopropanol in rats. *Fund. Appl. Toxicol.*, 22:152-158.

Beyer KK. 1992. Multi-Generation Rat Reproduction Study with Isopropanol, FINAL REPORT. Apr 17; Project No. 259835.

Brugnone F, Perbellini L, Apostoli P, Bellomi M, and Caretta D. 1983. Isopropanol exposure: environmental and biological monitoring in a printing works. *Br. J. Industrial Med.*, 40:160-168.

Burleigh-Flayer HD, Gill MW, Strother DE, Masten LW, McKee RH, Tyler TR, Gardiner T. 1994. Isopropanol 13-week vapor inhalation study in rats and mice with neurotoxicity evaluation in rats. *Fund. Appl. Toxicol.* 23:421-428.

C&EN. 1995. Chemical & Engineering News. Production by the U.S. chemical industry. M. Heylin ed., American Chemical Society, Washington D. C., June 26:38-44.

Guseinov VG, and Abasov DM. 1982. Toxicological characteristics of isopropyl alcohol after chronic exposure under experimental conditions. *Azerb. Med. Zh.* 58:53-57.

HSDB. 1995. Hazardous Substances Data Bank. National Library of Medicine, Bethesda, MD (CD-ROM version). Micromedex, Inc., Denver, CO (edition expires 11/31/95).

IARC. 1997. IARC monographs on the evaluation of the carcinogenic risk of chemicals to man. Vol. 15, pp. 223-243.

Laham S, Potvin M, Schrader K, and Marino I. 1980. Studies on inhalation toxicity of 2-propanol. *Drug Chem. Toxicol.*, 3(4):343-360.

Lehman AJ, Schwerma H, and Rickards E. 1945. Isopropyl alcohol acquired tolerance in dogs, rate of disappearance from the blood stream in various species, and effects on successive generation of rats. *J. Pharmacol. Exp. Ther.*, 85:61-69.

Nelson BK, Brightwell WS, MacKenzie-Taylor DR, Khan A, Burg JR, Weigel WW, and Goad PT. 1988. Teratogenicity of *n*-propanol and isopropanol administered at high inhalation concentrations to rats. *Fd. Chem. Toxicol.*, 26(3):247-254.

Phillips RD, and Cockrell B. 1984. Effect of certain light hydrocarbons on kidney function and structure in male rats. *Adv. Mod. Environ. Toxicol.*, 7:89-106.

Pilegaard K, and Ladefoged O. 1993. Toxic effects in rats of twelve weeks' dosing of 2-propanol, and neurotoxicity measured by densitometric measurements of glial fibrillary acidic protein in the dorsal hippocampus. *in vivo*, 7:325-330.

Savolainen H, Pekari K, and Helojoki H. 1979. Neurochemical and behavioural effects of extended exposure to isopropanol vapour with simultaneous ethanol intake. *Chem.-biol. Interact.*, 28:237-248.

Slauter R, Coleman D, Gaudette N, McKee R, Masten L, Gardiner T, Strother D, Tyler R, and Jeffcoat A. 1994. Disposition and pharmacokinetics of isopropanol in F-344 rats and B6C3F1 mice. *Fund. Appl. Toxicol.*, 23:407-420.

Teramoto K, Wakitani F, Horiguchi S, Jo T, Yamamoto T, Mitsutake H, and Nakseko H. 1993. Comparison of the neurotoxicity of several chemicals estimated by the peripheral nerve conduction velocity in rats. *Environ. Res.*, 62:148-154.

Tyl RW, Masten LW, Marr MC, Myers CB, Slauter RW, Gardiner TH, Strother DE, McKee RH, and Tyler TR. 1994. Developmental toxicity evaluation of isopropanol by gavage in rats and rabbits. *Fund. Appl. Toxicol.*, 22:139-151.

Union Carbide Corporation. 1990. Letter from Union Carbide Corp. to U.S. EPA containing toxicology info. for isopropanol with attachments and poster: Isopropanol ninety-day vapor inhalation neurotoxicity study in female Fischer 344 rats. EPA/OTS Doc 0890-0784.

United States Environmental Protection Agency/Office of Toxic Substances. 1986. A pilot one-generation study with isopropyl alcohol in rats (final report) with attachments and cover letter dated 013092. EPA/OTS Doc. #86-920000728. Prepared by British Industrial Biological Research Association. Submitted by Chemical Manufacturers Association. Fiche No. NTIS/OTS0535607.

United States Environmental Protection Agency/Office of Toxic Substances. 1992a. A teratology study with isopropyl alcohol in rats. Letter submitting one enclosed teratology study and one enclosed supplementary study on isopropanol. EPA/OTS Doc. #86-920000727. Prepared by British Industrial Biological Research Association. Submitted by Chemical Manufacturers Association. Fiche No. NTIS/OTS0535606.

United States Environmental Protection Agency/Office of Toxic Substances. 1992b. Investigations supplementary to studies with isopropyl alcohol. Letter submitting one enclosed teratology study and one enclosed supplementary study on isopropanol. EPA/OTS Doc. #86-920000727. Prepared by British Industrial Biological Research Association. Submitted by Chemical Manufacturers Association. Fiche No. NTIS/OTS0535604.

Wills JH, Jameson EM, and Coulston F. 1969. Effects on man of daily ingestion of small doses of isopropyl alcohol. *Toxicol. Appl. Pharmacol.*, 15:560-565.

CHRONIC TOXICITY SUMMARY

**MALEIC ANHYDRIDE**

(2,5-furandione; cis-butenedioic anhydride; toxilic anhydride; maleic andride)

**CAS Registry Number: 108-31-6**

**I. Chronic Toxicity Summary**

<i>Inhalation reference exposure level</i>	<b>0.2 µg/m<sup>3</sup></b>
<i>Critical effect(s)</i>	Hyperplastic change and/or neutrophilic infiltration of the nasal epithelium and irritation of the respiratory system of rats, hamsters, and monkeys
<i>Hazard index target(s)</i>	Respiratory system

**II. Chemical Property Summary (HSDB, 1995)**

<i>Molecular formula</i>	C <sub>4</sub> H <sub>2</sub> O <sub>3</sub>
<i>Molecular weight</i>	98.06 g/mol
<i>Description</i>	Colorless or white solid
<i>Vapor pressure</i>	0.1 torr @ 25°C (AIHA, 1970)
<i>Solubility</i>	Soluble in water, ether, acetate, chloroform, dioxane; @ 25°C, 227 g/100 g acetone, 112 g/100 g ethyl acetate, 52.5 g/100 g chloroform, 50 g/100 g benzene, 23.4 g/100 g toluene, 19.4 g/100 g o-xylene, 0.6 g/100 g CCl <sub>4</sub> , 0.25 g/100 g ligroin
<i>Conversion factor</i>	4.0 µg/m <sup>3</sup> per ppb at 25°C

**III. Major Uses and Sources**

Maleic anhydride is used as a chemical intermediate in the synthesis of fumaric and tartaric acid, certain agricultural chemicals, resins in numerous products, dye intermediates, and pharmaceuticals (HSDB, 1995). It is also used as a co-monomer for unsaturated polyester resins, an ingredient in bonding agents used to manufacture plywood, a corrosion inhibitor, and a preservative in oils and fats.

#### IV. Effects of Human Exposure

There are several case reports describing asthmatic responses possibly resulting from exposure to maleic anhydride. An individual showing an acute asthmatic reaction after exposure to dust containing maleic anhydride was described (Lee *et al.*, 1991). Concentrations of maleic anhydride in the inspirable particulate mass was  $0.83 \text{ mg/m}^3$  and in the respirable particulate mass was  $0.17 \text{ mg/m}^3$ . Bronchial provocation testing was performed with phthalic anhydride, lactose, and maleic anhydride. Exposure to maleic anhydride at  $0.83 \text{ mg/m}^3$  and  $0.09 \text{ mg/m}^3$  in inspirable and respirable particulate mass, respectively, showed a response of cough, rhinitis, and tearing within two minutes. Within 30 minutes, rales developed in both lungs and peak flow rate decreased 55%.

An individual occupationally exposed to maleic anhydride who developed wheezing and dyspnea upon exposure was described (Gannon *et al.*, 1992). After a period without exposure, two re-exposures both resulted in episodes of severe hemolytic anemia. There was no evidence of pulmonary hemorrhage. Radioallergosorbent testing showed specific IgE antibodies against human serum albumin conjugates with maleic anhydride, phthalic anhydride, and trimellitic anhydride, but not with tetrachlorophthalic anhydride. A critique of the Gannon *et al.* (1992) study (Jackson and Jones, 1993) questions the relationship of maleic anhydride exposure to the onset of the anemia, citing extended periods of exposure to maleic anhydride before symptoms appeared.

Another case report of occupational asthma due to exposure to maleic anhydride was described (Guerin *et al.*, 1980).

Humans exposed to maleic anhydride showed respiratory tract and eye irritation at concentrations of 0.25 to 0.38 ppm maleic anhydride (Grigor'eva, 1964). No irritation was reported at 0.22 ppm maleic anhydride.

#### V. Effects of Animal Exposure

Short *et al.* (1988) chronically exposed CD rats (15/sex/group), Engle hamsters (15/sex/group), and rhesus monkeys (3/sex/group) to maleic anhydride by inhalation. Four groups of each species were exposed to concentrations of 0, 1.1, 3.3, or  $9.8 \text{ mg/m}^3$  maleic anhydride for 6 hours/day, 5 days/week, for 6 months in stainless steel and glass inhalation chambers. Solid maleic anhydride was heated to  $53^\circ\text{C}$  to generate vapors which were then mixed with a stream of nitrogen. Chamber target levels were monitored by gas chromatography as total maleic (maleic anhydride plus maleic acid). No exposure-related increase in mortality occurred. Of the species examined, only rats showed significant changes in body weight during the course of the experiment, with reductions among males in the high-dose groups after exposure Day 40 and a transient weight reduction from Days 78-127 in the mid-dose group. Animals of all types exposed to any level of maleic anhydride showed signs of irritation of the nose and eyes, with nasal discharge, dyspnea, and sneezing reported frequently. No exposure-related eye abnormalities were reported. The severity of symptoms was reported to increase with increased

dose. No dose-related effects were observed in hematological parameters, clinical chemistry, or urinalysis. No effects on pulmonary function in monkeys were observed. Dose-related increases in the incidence of hyperplastic change in the nasal epithelium occurred in rats in all exposed groups, and in hamsters in the mid- and high-dose groups. Neutrophilic infiltration of the epithelium of the nasal tissue was observed in all species examined at all exposure levels. All changes in the nasal tissues were judged to be reversible. The only other significant histopathological observation was slight hemosiderin pigmentation in the spleens of female rats in the high-dose group.

The teratogenicity and multigeneration reproductive toxicity of maleic anhydride was also investigated (Short *et al.*, 1986). To evaluate teratogenicity, pregnant CD rats were treated orally with maleic anhydride in corn oil at concentrations of 0, 30, 90, or 140 mg/kg-day from gestational days 6-15. Animals were sacrificed on gestational day 20. No statistically significant dose-related effects were observed in maternal weight gain, implantation, fetal viability, post-implantation loss, fetal weight, or malformations. Groups of 10 male rats and 20 female rats/group (F<sub>0</sub> animals) were orally treated with 0, 20, 55, or 150 mg/kg-day maleic anhydride in corn oil to study multigeneration reproductive toxicity. Animals within the same dose group were bred together after 80 days of treatment to produce two F<sub>1</sub> generation animals (F<sub>1a</sub> and F<sub>1b</sub>) and animals from the F<sub>1</sub> generation were interbred to produce two F<sub>2</sub> generation animals (F<sub>2a</sub> and F<sub>2b</sub>). A significant increase in mortality was observed among both F<sub>0</sub> and F<sub>1</sub> generation animals in the high-dose group. Total body weight was significantly reduced in animals in the high-dose group at Week 11 of exposure for the F<sub>0</sub> generation males and females and at Week 30 of exposure in the F<sub>1</sub> generation males. No consistent pattern of dose- or treatment-related effect on fertility, litter size, or pup survival was observed. Examination of F<sub>0</sub> animals showed necrosis of the renal cortex in the high-dose group (60% of males and 15% of females). Absolute kidney weights were significantly increased in F<sub>1</sub> female in the low- and mid-dose groups, although there was no histological correlate. No changes in organ weight or histology were observed in the F<sub>2</sub> generation animals.

## VI. Derivation of Chronic Reference Exposure Level (REL)

<i>Study</i>	Short <i>et al.</i> , 1988
<i>Study population</i>	Rats (15/sex/group), hamsters (15/sex/group), and monkeys (3/sex/group)
<i>Exposure method</i>	Discontinuous inhalation exposure (0, 1.1, 3.3, or 9.8 mg/m <sup>3</sup> )
<i>Critical effects</i>	Hyperplastic change and neutrophilic infiltration of the nasal epithelium, respiratory irritation in all species
<i>LOAEL</i>	1.1 mg/m <sup>3</sup>
<i>NOAEL</i>	Not observed
<i>Exposure continuity</i>	6 hr/day, 5 days/week
<i>Average experimental exposure</i>	0.20 mg/m <sup>3</sup> for LOAEL group

<i>Human equivalent concentration</i>	0.019 mg/m <sup>3</sup> for LOAEL group (gas with extrathoracic respiratory effects, RGDR = 0.096, based on hamster data)
<i>Exposure duration</i>	6 months
<i>LOAEL uncertainty factor</i>	3
<i>Subchronic uncertainty factor</i>	1
<i>Interspecies uncertainty factor</i>	3
<i>Intraspecies uncertainty factor</i>	10
<i>Cumulative uncertainty factor</i>	100
<i>Inhalation reference exposure level</i>	0.0002 mg/m <sup>3</sup> (0.2 µg/m <sup>3</sup> , 0.00005 ppm, 0.05 ppb)

Short *et al.* (1988) examined the toxicity of maleic anhydride to rats, hamsters, and monkeys by the inhalation route of exposure. Dose- and exposure related effects, although mild and reversible, were observed at all exposure levels. Specifically, exposure to maleic anhydride vapors resulted in hyperplastic change to the nasal epithelium of rats and hamsters (obligate nose breathers) and neutrophilic infiltration of the nasal epithelium was observed in all three species at all levels of exposure. All species also showed signs of irritation at all exposure levels. The observation that acute maleic anhydride is a strong respiratory irritant to humans (ACGIH, 1992) suggests this is a valid endpoint of toxicity to humans as well. Human exposure at levels as low as ~1 mg/m<sup>3</sup> appears to trigger acute asthmatic reactions in sensitive individuals (Lee *et al.*, 1991). Although there is no evidence of a toxic response similar to the development of asthma in animals, the 1.1 mg/m<sup>3</sup> LOAEL from the animal studies of Short *et al.* (1988) results in a REL which should protect asthmatics.

The major strengths of the REL are the availability of multiple-species, multiple-dose subchronic inhalation studies and the observation of a mild effect LOAEL. The major uncertainties are the lack of human data and the lack of a NOAEL observation.

## VII. References

- ACGIH. 1992. American Conference of Governmental Industrial Hygienists, Inc. Documentation of the threshold limit values and biological exposure indices. Sixth edition. Cincinnati, OH.
- AIHA. 1970. American Industrial Hygiene Association. *Am Ind Hyg Assoc J*, 31:391-4.
- Gannon PFG, Burge PS, Hewlett C, and Tee RD. 1992. Haemolytic anaemia in a case of occupational asthma due to maleic anhydride. *Br J Ind Med*, 49:142-3.
- Grigor'eva, K.V. 1964. Pollution of atmospheric air with maleic anhydride (Abstract in *Chem Abstr* (1966) 65:14319b). *Gig Sanit*, 29:7-11.

Guerin JC, Deschamps O, Guillot TL, Chavallion JM, and Kalb JC. 1980. A propos d'un cas d'asthme a l'anhydride maleique [A case of asthma due to maleic anhydride]. Poumon et le Coeur, 36:393-5.

HSDB. 1995. Hazardous Substances Data Bank. National Library of Medicine, Bethesda, Maryland (CD-ROM Version). Micromedex, Inc., Denver, Colorado (Edition expires 7/31/96).

Jackson J, and Jones AH. 1993. Correspondence: Haemolytic anaemia in a case of occupational asthma due to maleic anhydride. (with reply by P. Gannon). Br J Ind Med, 50:191-2.

Lee HS, Wang YT, Cheong TH, Tan KT, Chee BE, and Narendran K. 1991. Occupational asthma due to maleic anhydride. Br J Ind Med, 48:283-5.

Short RD, Johannsen FR, Levinskas GJ, Rodwell DE, and Schardein JL. 1986. Teratology and multigeneration reproduction studies with maleic anhydride in rats. Fundam Appl Toxicol, 7:359-66.

Short RD, Johannsen FR, and Ulrich CE. 1988. A 6-month multispecies inhalation study with maleic anhydride. Fundam Appl Toxicol, 10:517-24.

CHRONIC TOXICITY SUMMARY

# MANGANESE AND COMPOUNDS

<i>Molecular Formula</i>	<i>Synonyms</i>	<i>Molecular Weight</i>	<i>CAS Reg. No.</i>
Mn	elemental manganese; colloidal manganese; cutaval	54.94 g/mol	7439-96-5
MnO	manganese oxide; manganese monoxide; manganosite	70.94 g/mol	1344-43-0
MnO <sub>2</sub>	manganese dioxide; black manganese oxide	86.94 g/mol	1313-13-9
Mn <sub>3</sub> O <sub>4</sub>	manganese tetroxide; trimanganese tetraoxide; manganomanganic oxide	228.82 g/mol	1317-35-7
MnCl <sub>2</sub>	manganese chloride; manganese dichloride; manganous chloride	125.84 g/mol	7773-01-5

## I. Chronic Toxicity Summary

<i>Inhalation reference exposure level</i>	<b>0.05 µg/m<sup>3</sup></b> (U.S. EPA RfC) This document summarizes the evaluation of non-cancer health effects by U.S. EPA for the RfC
<i>Critical effect(s)</i>	Impairment of neurobehavioral function in humans
<i>Hazard index target(s)</i>	Nervous system

## II. Physical and Chemical Properties (HSDB, 1995)

<i>Molecular formula</i>	See above
<i>Molecular weight</i>	See above
<i>Description</i>	Lustrous, gray-pink metal (Mn); green (MnO), black (MnO <sub>2</sub> ) or pink (MnCl <sub>2</sub> ) crystals; brownish-black powder (Mn <sub>3</sub> O <sub>4</sub> )
<i>Specific gravity</i>	7.21-7.4 (Mn - depending on allotropic form); 5.43-5.46 (MnO); 4.88 (Mn <sub>3</sub> O <sub>4</sub> ); 2.977 @ 25°C/4°C (MnCl <sub>2</sub> )
<i>Boiling point</i>	1962°C (Mn); not available (MnO); unknown (Mn <sub>3</sub> O <sub>4</sub> ); 1190°C (MnCl <sub>2</sub> )
<i>Melting point</i>	1244 ± 3°C (Mn); 1650°C (MnO); 2847°C (Mn <sub>3</sub> O <sub>4</sub> - NIOSH Pocket Guide <sup>TM</sup> , 1995); 650°C (MnCl <sub>2</sub> )
<i>Vapor pressure</i>	1 mm Hg @ 1292°C (Mn); 0 mm Hg (Mn <sub>3</sub> O <sub>4</sub> );

<i>Solubility</i>	not available (MnO; MnCl <sub>2</sub> ) Sol. in dil. acids and aq. solns. of Na- or K- bicarbonate (Mn); sol. in NH <sub>4</sub> Cl, insol. in H <sub>2</sub> O (MnO); insol. in H <sub>2</sub> O, HNO <sub>3</sub> , or cold H <sub>2</sub> SO <sub>4</sub> (MnO <sub>2</sub> - Reprotext®, 1995); insol. in H <sub>2</sub> O, sol. in HCl (Mn <sub>3</sub> O <sub>4</sub> ); 72.3 g/100 ml H <sub>2</sub> O @ 25°C (MnCl <sub>2</sub> )
<i>Conversion factor</i>	Not applicable (dusts or powders)

### III. Major Uses or Sources

Metallic manganese is used in the manufacturing of steel, carbon steel, stainless steel, cast iron, and superalloys to increase hardness, stiffness, and strength (HSDB, 1995). Manganese chloride is used in dyeing, disinfecting, batteries, and as a paint drier and dietary supplement. Manganese oxide (MnO) is used in textile printing, ceramics, paints, colored glass, fertilizers, and as food additives. Manganese dioxide is used in batteries and may also be generated from the welding of manganese alloys. Manganese tetroxide may be generated in situations where other oxides of manganese are heated in air (NIOSH Pocket Guide, 1995).

### IV. Effects of Human Exposure

Male workers (n=92, plus 101 matched controls) in an alkaline battery plant in Belgium exposed to manganese dioxide were the subject of a cross-sectional epidemiological investigation (Roels *et al.*, 1992). Evaluation of the subjects included tests for neurobehavioral function, lung function, hematological parameters, and urinalysis. Exposed workers showed significant differences in performance on tests of visual reaction time, eye-hand coordination, and hand tremor. Occupational-lifetime integrated respiratory dust (IRD) levels ranged from 0.04-4.43 mg Mn/m<sup>3</sup>-yr with a geometric mean of 0.793 mg Mn/m<sup>3</sup>-yr. Average exposure time was 5.3 years, with a range of 0.2-17.7 years. The authors grouped the workers into three exposure groups based on the IRD levels: <0.6, 0.6-1.2, and >1.2 mg Mn/m<sup>3</sup>-yrs. Although there was an indication of a linear dose-related trend for visual reaction time and hand steadiness, the authors concluded that "analysis of the data on a group basis...does not permit us to identify a threshold effect level for airborne Mn." A daily average exposure level of 0.15 mg Mn/m<sup>3</sup> was derived by dividing the geometric mean of the IRD (0.793 mg Mn/m<sup>3</sup>-yr) by the average exposure time (5.3 yr).

In an earlier study, 141 male workers plus 104 matched control workers were examined for effects of exposure to MnO<sub>2</sub>, manganese tetroxide (Mn<sub>3</sub>O<sub>4</sub>), and other manganese salts (Roels *et al.*, 1987). Tests measuring visual reaction time, eye-hand coordination, hand tremor, and short-term memory were found to be significantly different in the manganese-exposed group. Statistically significant clinical symptoms (as evaluated in a questionnaire) included fatigue, tinnitus, finger trembling and irritability. Self-reported prevalence of coughs, colds and acute bronchitis were increased in the manganese exposed group relative to controls. Mean time of

employment was 7.1 years with a range of 1-19 years. Total airborne manganese dust levels had an arithmetic mean of 1.33 mg/m<sup>3</sup> and a geometric mean of 0.94 mg/m<sup>3</sup>.

Several other studies have identified neurobehavioral endpoints of manganese toxicity in human populations. A matched-pair cross-sectional study of 74 pairs of manganese alloy workers (Mergler *et al.*, 1994). Matched pairs were found to be discordant in reporting a number of adverse clinical symptoms including the following areas: fatigue, emotional state, memory, attention, concentration difficulty, nightmares, unusual sweating, sexual dysfunction, lower back pain, joint pain, and tinnitus. Motor function tests also revealed deficits in the manganese exposed group. Olfactory perception was enhanced in the manganese exposed group. Exposure levels were estimated at a geometric mean of 0.035 mg Mn/m<sup>3</sup> for respirable dust and 0.225 mg Mn/m<sup>3</sup> for total dust. Mean duration of exposure was 16.7 years.

Workers in two Swedish foundries were evaluated for potential neurobehavioral effects from exposure to manganese (Iregren, 1990). Exposure levels ranged from 0.02-1.4 mg Mn/m<sup>3</sup> with a mean of 0.25 mg Mn/m<sup>3</sup>. Simple reaction time, standard deviation of reaction time, finger-tapping speed, digit-span short term memory, speed of mental addition, and verbal understanding were significantly different from controls among manganese exposed workers.

Further reporting of the workers described by Iregren *et al.* (1990) evaluated more neurobehavioral and electrophysiological endpoints of toxicity from manganese exposure (Wennberg *et al.*, 1991; Wennberg *et al.*, 1992). Although many of the parameters measured showed differences (increased self-reported health symptoms, increased abnormal EEGs, abnormal extrapyramidal function), these results were not statistically significant.

The workers reported on by Roels *et al.* (1987) were examined for potential reproductive toxicity (Lauwerys *et al.*, 1985). These investigators found that for workers divided into certain age groups (16-25 and 26-35), there was a decrease in the number of children born to these workers.

Evaluation of reproductive toxicity of the workers reported on by Roels *et al.* (1992) showed no difference in the probability of live birth in a comparison of manganese exposed workers with controls (Gennart *et al.*, 1992). Comparison of reproductive hormones (FSH, LH, prolactin) also showed no differences between the groups.

Junior high school students exposed to manganese were examined for potential effects on the respiratory system (Nogawa *et al.*, 1973). Measurement of atmospheric manganese levels showed a 5-day average level of 0.0067 mg Mn/m<sup>3</sup> 300 m from the school.

## V. Effects of Animal Exposure

Toxic effects have been described in animals exposed to manganese compounds by inhalation (Shiotsuka, 1984; Suzuki *et al.*, 1978; Moore *et al.*, 1975). Shiotsuka *et al.* (1984) demonstrated increased incidence of pneumonia among rats exposed for 2 weeks to manganese dioxide concentrations ranging from 68-219 mg/m<sup>3</sup>. Monkeys exposed to manganese dioxide concentrations ranging from 0.7-3.0 mg/m<sup>3</sup> for 10 months showed increased incidence of pulmonary emphysema (Suzuki *et al.*, 1978) and hamsters and rats exposed for 56 days to 0.117 mg Mn<sub>3</sub>O<sub>4</sub>/m<sup>3</sup> showed bronchial lesions (Moore *et al.*, 1975).

High concentrations of manganese (>10 mg/m<sup>3</sup>) have decreased host resistance in exposed animals (Adkins *et al.*, 1980; Bergstrom, 1977; Maigetter *et al.*, 1976).

Nine month inhalation toxicity studies in rats and monkeys exposed to levels as high as 1.15 mg Mn<sub>3</sub>O<sub>4</sub>/m<sup>3</sup> produced no significant pulmonary effects (Ulrich *et al.*, 1979a; Ulrich *et al.*, 1979b; Ulrich *et al.*, 1979c)

## VI. Derivation of U.S. EPA Reference Concentration

### *Derivation of U.S. EPA Reference Concentration*

<i>Study</i>	Roels <i>et al.</i> , 1992 (evaluated by U.S. EPA, 1993)
<i>Study population</i>	Occupationally-exposed humans
<i>Exposure method</i>	Discontinuous occupational inhalation exposure to manganese dioxide (0.2, 1.0, and 6.0 mg/m <sup>3</sup> )
<i>Critical effects</i>	Impairment of neurobehavioral function
<i>LOAEL</i>	0.15 mg respirable manganese dust/m <sup>3</sup> (geometric mean from exposures of 0.040 to 4.4 mg Mn/m <sup>3</sup> -years)
<i>NOAEL</i>	Not observed
<i>Study continuity</i>	8 hours per day, 5 days per week
<i>Average occupational exposure</i>	0.05 mg/m <sup>3</sup> for LOAEL group (based on an 8-hour TWA occupational exposure to 10 m <sup>3</sup> manganese contaminated air per day out of 20 m <sup>3</sup> total air inhaled per day over 5 days per week)
<i>Human equivalent concentration</i>	0.05 mg/m <sup>3</sup> for LOAEL group
<i>Study duration</i>	5.3 years (average)
<i>LOAEL uncertainty factor</i>	10
<i>Subchronic uncertainty factor</i>	3
<i>Interspecies uncertainty factor</i>	1
<i>Intraspecies uncertainty factor</i>	10
<i>Modifying factor</i>	3 (lack of developmental data and potential differences in toxicity for different forms of

	manganese)
<i>Cumulative uncertainty factor</i>	1,000
<i>Inhalation reference exposure level</i>	0.05 µg/m <sup>3</sup>

In the derivation of its reference concentration for manganese and compounds, the U.S. EPA selected the Roels *et al.* (1992) study for establishing the exposure level associated with adverse health effects. Although this study did not establish a no adverse effect level (NOAEL), clear evidence of toxicity was established at the level of exposure which was found in the facility studied, and was therefore taken to be a LOAEL. Advantages offered by this study over the other available studies of manganese toxicity include: (1) the study population was human, (2) the workers were only exposed to a single manganese compound, (3) the study population was well controlled for with matching for age, height, weight, work schedule, coffee and alcohol consumption, and smoking, (4) the exposure duration was relatively long and work practice continuity suggests exposure levels changed little over time, and (5) the effects observed were consistent with those observed among other workers occupationally exposed to manganese.

The strengths of the inhalation REL include the use of human exposure data from workers exposed over a period of years. Major areas of uncertainty are the lack of observation of a NOAEL, the uncertainty in estimating exposure and the potential variability in exposure concentration, the lack of chronic inhalation exposure studies, and the lack of reproductive and developmental toxicity studies.

#### ***Derivation of U.S. EPA Reference Dose***

<i>Study</i>	Freeland-Graves <i>et al.</i> , 1987; NRC, 1989; WHO, 1973
<i>Study population</i>	Various human populations
<i>Exposure method</i>	Chronic ingestion of foodstuffs
<i>Critical effects</i>	CNS effects
<i>LOAEL</i>	Not determined
<i>NOAEL</i>	0.14 mg/kg-day
<i>Exposure continuity</i>	
<i>Exposure duration</i>	Chronic
<i>Average experimental exposure</i>	Up to lifetime
<i>LOAEL uncertainty factor</i>	1
<i>Subchronic uncertainty factor</i>	1
<i>Interspecies uncertainty factor</i>	1
<i>Intraspecies factor</i>	1
<i>Cumulative uncertainty factor</i>	1
<i>Oral reference exposure level</i>	0.14 mg/kg-day (U.S. EPA RfD)

\*Conversion Factors and Assumptions -- The NOAEL of 10 mg/day (0.14 mg/kg-day for 70 kg adult) for chronic human consumption of manganese in the diet is based on a composite of data from several studies.

The oral Reference Exposure Level (REL) for manganese is the U.S. EPA's Oral RfD (IRIS, 1996). The principal studies used were: (1) Freeland-Graves, J.H., C.W. Bales and F. Behmardi. 1987. Manganese requirements of humans. In: Nutritional Bioavailability of Manganese, C. Kies, ed. American Chemical Society, Washington, DC. p. 90-104. (2) NRC (National Research Council). 1989. Recommended Dietary Allowances, 10th ed. Food and Nutrition Board, National Research Council, National Academy Press, Washington, DC. p. 230-235. and (3) WHO (World Health Organization). 1973. Trace Elements in Human Nutrition: Manganese. Report of a WHO Expert Committee. Technical Report Service, 532, WHO, Geneva, Switzerland. p. 34-36.

Manganese is a ubiquitous element that is essential for normal physiologic functioning in all animal species. Several disease states in humans have been associated with both deficiencies and excess intakes of manganese. Thus any quantitative risk assessment for manganese must take into account aspects of both the essentiality and the toxicity of manganese. In humans, many data are available providing information about the range of essentiality for manganese. In addition, there are many reports of toxicity to humans exposed to manganese by inhalation; much less is known, however, about oral intakes resulting in toxicity. Rodents do not provide a good experimental model for manganese toxicity, and only one limited study in primates by the oral route of exposure is available. The following assessment, therefore, focuses more on what is known to be a safe oral intake of manganese for the general human population. Finally, it is important to emphasize that individual requirements for, as well as adverse reactions to, manganese may be highly variable. The reference dose is estimated to be an intake for the general population that is not associated with adverse health effects; this is not meant to imply that intakes above the reference dose are necessarily associated with toxicity. Some individuals may, in fact, consume a diet that contributes more than 10 mg Mn/day without any cause for concern.

The Food and Nutrition Board of the National Research Council (NRC, 1989) determined an "estimated safe and adequate daily dietary intake" (ESADDI) of manganese to be 2-5 mg/day for adults. The lower end of this range was based on a study by McLeod and Robinson (1972), who reported equilibrium or positive balances at intakes of 2.5 mg Mn/day or higher. The range of the ESADDI also includes an "extra margin of safety" from the level of 10 mg/day, which the NRC considered to be safe for an occasional intake. While the NRC determined an ESADDI for manganese of 2-5 mg/day, some nutritionists feel that this level may be too low. Freeland-Graves *et al.* (1987) have suggested a range of 3.5-7 mg/day for adults based on a review of human studies. It is noted that dietary habits have evolved in recent years to include a larger proportion of meats and refined foods in conjunction with a lower intake of whole grains. The net result of such dietary changes includes a lower intake of manganese such that many individuals may have suboptimal manganese status.

The World Health Organization (WHO, 1973) reviewed several investigations of adult diets and reported the average daily consumption of manganese to range from 2.0-8.8 mg Mn/day. Higher manganese intakes are associated with diets high in whole-grain cereals, nuts, green leafy vegetables, and tea. From manganese balance studies, the WHO concluded that 2-3 mg/day is adequate for adults and 8-9 mg/day is "perfectly safe." Evaluations of standard diets from the

United States, England, and Holland reveal average daily intakes of 2.3-8.8 mg Mn/day. Depending on individual diets, however, a normal intake may be well over 10 mg Mn/day, especially from a vegetarian diet. While the actual intake is higher, the bioavailability of manganese from a vegetarian diet is lower, thereby decreasing the actual absorbed dose.

From this information taken together, EPA concludes that an appropriate reference dose for manganese is 10 mg/day (0.14 mg/kg-day). In applying the reference dose for manganese to a risk assessment, it is important that the assessor consider the ubiquitous nature of manganese, specifically that most individuals will be consuming about 2-5 mg Mn/day in their diet. This is particularly important when one is using the reference dose to determine acceptable concentrations of manganese in water and soils. There is one epidemiologic study of manganese in drinking water, performed by Kondakis *et al.* (1989). Three areas in northwest Greece were chosen for this study, with manganese concentrations in natural well water of 3.6-14.6 µg/L in area A, 81.6-252.6 µg/L in area B, and 1600-2300 µg/L in area C. The total population of the three areas studied ranged from 3200 to 4350 people. The study included only individuals over the age of 50 drawn from a random sample of 10% of all households (n=62, 49 and 77 for areas A, B and C, respectively). The authors reported that "all areas were similar with respect to social and dietary characteristics," but few details were reported. The three areas are located within a 200-square km region. Although the amount of manganese in the diet was not reported, the authors indicated that most of the food was purchased from markets and is expected to be comparable for all three areas. Chemicals other than manganese in the well water were reported to be within Economic Community (EC) standards, except for hardness (120-130 mg calcium carbonate per liter). The individuals chosen were submitted to a neurologic examination, the score of which represents a composite of the presence and severity of 33 symptoms (e.g., weakness/fatigue, gait disturbances, tremors, dystonia). Whole blood and hair manganese concentrations also were determined. The mean concentration of manganese in hair was 3.51, 4.49 and 10.99 µg/g dry weight for areas A, B and C, respectively ( $p < 0.0001$  for area C versus A). The concentration of manganese in whole blood did not differ between the three areas, but this is not considered to be a reliable indicator of manganese exposure. The mean ( $\bar{x}$ ) and range ( $r$ ) of neurologic scores were as follows: Area A (males:  $\bar{x} = 2.4$ ,  $r = 0-21$ ; females:  $\bar{x} = 3.0$ ,  $r = 0-18$ ; both  $\bar{x} = 2.7$ ,  $r = 0-21$ ); Area B (males  $\bar{x} = 1.6$ ,  $r = 0-6$ ; females:  $\bar{x} = 5.7$ ,  $r = 0-43$ ; both:  $\bar{x} = 3.9$ ,  $r = 0-43$ ); and Area C (males:  $\bar{x} = 4.9$ ,  $r = 0-29$ ; females:  $\bar{x} = 5.5$ ,  $r = 0-21$ ; both  $\bar{x} = 5.2$ ,  $r = 0-29$ ). The authors indicate that the difference in mean scores for area C versus A was significantly increased (Mann-Whitney  $z = 3.16$ ,  $p = 0.002$  for both sexes combined). In a subsequent analysis, logistic regression indicated that there is a significant difference between areas A and C even when both age and sex are taken into account (Kondakis, 1990).

The individuals examined in the Kondakis study also had exposure to manganese in their diet. This was originally estimated to be 10-15 mg/day because of the high intake of vegetables (Kondakis, 1990). This estimate was subsequently lowered to 5-6 mg/day (Kondakis, 1993). Because of the uncertainty in the amount of manganese in the diet and the amount of water consumed, it is impossible to estimate the total oral intake of manganese in this study. These limitations preclude the use of this study to determine a quantitative dose-response relationship for the toxicity of manganese in humans.

This study, nevertheless, raises significant concerns about possible adverse neurological effects at doses not far from the range of essentially. Because of this concern, it is recommended that a modifying factor of 3 be applied when assessing risk from manganese in drinking water or soil.

The information used to determine the RfD for manganese was taken from many large populations consuming normal diets over an extended period of time with no adverse health effects. As long as physiologic systems are not overwhelmed, humans exert an efficient homeostatic control over manganese such that body burdens are kept constant with variation in the manganese content of the diet. The information providing a chronic NOAEL in many cross-sections of human populations, taken in conjunction with the essentiality of manganese, warrants an uncertainty factor of 1.

When assessing exposure to manganese from food, the modifying factor is 1; however, when assessing exposure to manganese from drinking water or soil, a modifying factor of 3 is recommended. There are four reasons for this recommendation. First, while the data suggest that there is no significant difference between absorption of manganese as a function of the form in which it is ingested (i.e., food versus water), there is some degree of increased uptake of manganese from water in fasted individuals. Second, the study by Kondakis *et al.* (1989) raises some concern for possible adverse health effects associated with a lifetime consumption of drinking water containing about 2 mg/L of manganese. Third, although toxicity has not been demonstrated, there is concern for infants fed formula that typically has a much higher concentration of manganese than does human milk. If powdered formula is made with drinking water, the manganese in the water would represent an additional source of intake. Finally, there is some evidence that neonates absorb more manganese from the gastrointestinal tract, that neonates are less able to excrete absorbed manganese, and that in the neonate the absorbed manganese more easily passes the blood-brain barrier. These findings may be related to the fact that manganese in formula is in a different ionic form and a different physical state than in human milk. These considerations concerning increased exposure in an important population group, in addition to the likelihood that any adverse neurological effects of manganese are likely to be irreversible and not manifested for many years after exposure, warrant caution until more definitive data are available.

U.S. EPA stated its confidence in the RfC as: Study - Medium; Data Base - Medium; and RfD - Medium. Many studies have reported similar findings with regard to the normal dietary intake of manganese by humans. These data are considered to be superior to any data obtained from animal toxicity studies, especially as the physiologic requirements for manganese vary quite a bit among different species, with man requiring less than rodents. There is no single study used to derive the dietary RfD for manganese. While several studies have determined average levels of manganese in various diets, no quantitative information is available to indicate toxic levels of manganese in the diet of humans. Because of the homeostatic control humans maintain over manganese, it is generally not considered to be very toxic when ingested with the diet. It is important to recognize that while the RfD process involves the determination of a point estimate of an oral intake, it is also stated that this estimate is associated “with uncertainty spanning perhaps an order of magnitude.” Numerous factors, both environmental factors (e.g., the presence or absence of many dietary constituents) and biological or host factors (e.g., age,

alcohol consumption, anemia, liver function, general nutritional status) can significantly influence an individual's manganese status. As discussed in the Additional Studies / Comments Section, there is significant variability in the absorption and elimination of manganese by humans. Confidence in the data base is medium and confidence in the dietary RfD for manganese is also medium.

## VII. References

- Adkins B, Luginbuhl GH, Miller FJ, and Gardner DE. 1980. Increased pulmonary susceptibility to streptococcal infection following inhalation of manganese oxide. *Environ Res*, 23:110-20.
- Bergstrom R. 1977. Acute pulmonary toxicity of manganese dioxide. *Scand J Work Environ Health*, 3:1-40.
- Gennart J-P, Buchet J-P, Roels H, Ghyselen P, Ceulemans E, and Lauwerys R. 1992. Fertility of male workers exposed to cadmium, lead, or manganese. *Am J Epidemiol*, 135:1208-19.
- HSDB. 1995. Hazardous Substances Data Bank. National Library of Medicine, Bethesda, Maryland (CD-ROM Version). Micromedex, Inc., Denver, Colorado (Edition expires 7/31/96).
- Iregren A. 1990. Psychological test performance in foundry workers exposed to low levels of manganese. *Neurotoxicol Teratol*, 12:673-5.
- Lauwerys R, Roels H, Genet P, Toussaint G, Bouckaert A, and de Cooman S. 1985. Fertility of male workers exposed to mercury vapor or to manganese dust: A questionnaire study. *Am J Ind Med*, 7:171-6.
- Maigetter RZ, Ehrlich R, Fenter JD, and Gardner DE. 1976. Potentiating effects of manganese dioxide on experimental respiratory infections. *Environ Res*, 11:386-91.
- Mergler D, Huel G, Bowler R, Iregren A, Belanger S, Baldwin M, Tardif R, Smargiassi A, and Martin L. 1994. Nervous system dysfunction among workers with long-term exposure to manganese. *Environ Res*, 64:151-80.
- Moore W, Hysell D, Miller R, Malanchuk M, Hinnens R, Yang Y, and Stara JF. 1975. Exposure of laboratory animals to atmospheric manganese from automotive emissions. *Environ Res*, 9:274-84.
- NIOSH Pocket Guide<sup>TM</sup> 1995. National Institute of Occupational Safety and Health. National Library of Medicine, Bethesda, Maryland (CD-ROM Version). Micromedex, Inc., Denver, Colorado (Edition expires 7/31/96).

Nogawa K, Kobayashi E, Sakamoto M, *et al.* 1973. [Studies of the effects on the respiratory organs of air pollution consisting of dusts composed mainly of manganese. (First report). Effects on the respiratory organs of junior high school students]. *Nippon Koshu Eisei Zasshi*, 20:315-25. Reprotext® 1995. National Library of Medicine, Bethesda, Maryland (CD-ROM Version). Micromedex, Inc., Denver, Colorado (Edition expires 7/31/96).

Roels HA, Ghyselen P, Buchet JP, Ceulemans E, and Lauwerys RR. 1992. Assessment of the permissible exposure level to manganese in workers exposed to manganese dioxide dust. *Br J Ind Med*, 49:25-34.

Roels H, Lauwerys R, Buchet JP, Genet P, Sarhan MJ, Hanotiau I, de Fays M, Bernard A, and Stanescu D. 1987. Epidemiological survey among workers exposed to manganese: Effects on lung, central nervous system, and some biological indices. *Am J Ind Med*, 11:307-27.

Shiotsuka RN. 1984. Inhalation toxicity of manganese dioxide and a magnesium oxide-manganese dioxide mixture. Inhalation Toxicology Facility, Brookhaven National Laboratory, Upton, NY. BNL 35334.

Suzuki Y, Fujii N, Yano H, Ohkita T, Ichikawa A, and Nishiyama K. 1978. Effects of the inhalation of manganese dioxide dust on monkey lungs. *Tokushima J Exp Med*, 25:119-25.

Ulrich CE, Rinehart W, and Brandt M. 1979c. Evaluation of the chronic inhalation toxicity of a manganese oxide aerosol. III. Pulmonary function, electromyograms, limb tremor, and tissue manganese data. *Am Ind Hyg Assoc J*, 40:349-53.

Ulrich CE, Rinehart W, and Busey W. 1979a. Evaluation of the chronic inhalation toxicity of a manganese oxide aerosol. I. Introduction, experimental design, and aerosol generation methods. *Am Ind Hyg Assoc J*, 40:238-44.

Ulrich CE, Rinehart W, Busey W, and Dorato MA. 1979b. Evaluation of the chronic inhalation toxicity of a manganese oxide aerosol. II. Clinical observations, hematology, clinical chemistry and histopathology. *Am Ind Hyg Assoc J*, 40:322-9.

U.S. EPA. 1993. United States Environmental Protection Agency. Documentation of the reference concentration for chronic inhalation exposure (RfC) for manganese. Integrated Risk Information System (IRIS on-line). U.S. EPA: Washington, DC.

Wennberg A, Hagman M, and Johansson L. 1992. Preclinical neurophysiological signs of parkinsonism in occupational manganese exposure. *Neurotoxicology*, 13:271-4.

Wennberg A, Iregren A, Struwe G, Cizinsky G, Hagman M, and Johansson L. 1991. Manganese exposure in steel smelters a health hazard to the nervous system. *Scand J Work Environ Health*, 17:255-62.

*CHRONIC TOXICITY SUMMARY*

## INORGANIC MERCURY

*(liquid silver; hyfarargyrum; colloidal mercury)*

**CAS Registry Number: 7439-97-6**

### I. Chronic Toxicity Summary

<i>Inhalation reference exposure level</i>	<b>0.3 <math>\mu\text{g}/\text{m}^3</math></b> (U.S. EPA RfC) This document summarizes the evaluation of non-cancer health effects by U.S. EPA for the RfC
<i>Critical effects</i>	Hand tremor, memory disturbances, and autonomic dysfunction in humans
<i>Hazard index target(s)</i>	Nervous system

### II. Chemical Property Summary (HSDB, 1995)

<i>Molecular formula</i>	Hg
<i>Molecular weight</i>	200.59 g/mol
<i>Description</i>	Silvery, odorless, heavy liquid
<i>Vapor pressure</i>	0.002 mm Hg @ 25°C
<i>Solubility</i>	Soluble in concentrated nitric and hot sulfuric acids; dissolves to some extent in lipids
<i>Conversion factor</i>	1 ppm = 8.2 $\text{mg}/\text{m}^3$

### III. Major Uses or Sources

The uses of mercury and mercury containing compounds are considerable. Because it has uniform volume expansion with increasing temperature over the entire temperature range of its liquid state it is widely used in barometers, thermometers, hydrometers, and pyrometers. It is used in mercury arc lamps producing ultraviolet rays, in fluorescent lamps, as a catalyst in oxidation of organic compounds, extracting gold and silver from ores, electric rectifiers, the making of mercury fulminate, for Millon's Reagent, and as a cathode in electrolysis. It is also used in pulp and paper manufacturing, as a component of batteries, in amalgams (dental preparations), and in the manufacture of switching devices such as oscillators, the manufacture of chlorine and caustic soda, as a lubricant, and a laboratory reagent.

To lesser extent it has been used to fumigate and protect grain from insect infestation, in pharmaceuticals, agricultural chemicals, and antifouling paints (ACGIH, 1991).

#### IV. Effect of Exposure to Humans

The primary effects of chronic exposure to mercury vapor are on the central nervous system. Chronic duration exposures to elemental mercury have resulted in tremors (mild or severe), unsteady walking, irritability, poor concentration, short-term memory deficits, tremulous speech, blurred vision, performance decrements, parasthesia, and decreased nerve conduction (Albers *et al.*, 1988; Chaffin *et al.*, 1977; Fawer *et al.*, 1983; Kishi *et al.*, 1993; Langolf *et al.*, 1978; Piikivi *et al.*, 1984; Smith *et al.*, 1970). Motor system disturbance can be reversible upon cessation of exposure, however, memory deficits may be permanent (Chaffin *et al.*, 1973). Studies have shown effects such as tremor and decreased cognitive skills in workers exposed to approximately 25  $\mu\text{g}/\text{m}^3$  mercury vapor (Piikivi *et al.*, 1984; Piikivi and Hanninen, 1989; Piikivi and Toulonen, 1989) (see discussion below).

The kidney is also a sensitive target organ of mercury toxicity. Effects such as proteinuria, proximal tubular and glomerular changes, albuminuria, glomerulosclerosis, and increased urinary N-acetyl-B-glucosaminidase have been seen in workers exposed to approximately 25-60  $\mu\text{g}/\text{m}^3$  mercury vapor (Barregard *et al.*, 1988; Bernard *et al.*, 1987; Roels *et al.*, 1982; Piikivi and Ruukonen, 1989).

Chronic exposure to mercury vapors has also resulted in cardiovascular effects such as increased heart and blood pressure (Fagala and Wigg, 1992; Taueg *et al.*, 1992; Piikivi, 1989) and has produced leukocytosis and neutrophilia (Fagala and Wigg, 1992).

A number of other studies with similar exposure levels also found adverse psychological and neurological effects in exposed versus unexposed individuals. Piikivi and Tolonen (1989) used EEGs to study the effects of long-term exposure to mercury vapor in 41 chloralkali workers exposed for a mean of 15.6 years as compared to matched controls. They found that exposed workers who had blood mercury levels of 12  $\mu\text{g}/\text{L}$  tended to have an increased number of EEG abnormalities when analyzed by visual inspection. When analyzed by computer, brain activity was found to be significantly lower than matched controls. The changes were most prominent in the parietal cortex, but absent in the frontal cortex.

Another study by Piikivi (1989) examined subjective and objective symptoms of autonomic dysfunction in 41 chloralkali workers exposed to mercury vapor for an average of 15.6 years as compared with matched controls. Similar to the above studies, the exposed workers had mean blood levels of 11.6  $\mu\text{g}/\text{L}$  corresponding to a TWA exposure of 25  $\mu\text{g Hg}/\text{m}^3$  in air (Roels *et al.*, 1987). The workers were tested for pulse rate variation in normal and deep breathing, the Valsava maneuver, vertical tilt, and blood pressure responses during standing and isometric work. The only significant difference in subjective symptoms was an increased reporting of palpitations in exposed workers. The objective tests demonstrated an increase in pulse rate variations at 30  $\mu\text{g Hg}/\text{m}^3$  (extrapolated from blood level based on methods of Roel *et al.* (1987)), which is indicative of autonomic reflex dysfunction.

A more recent study by Ngim *et al.* (1992) assessed neurobehavioral performance in a cross-sectional study of 98 dentists exposed to a TWA concentration of  $14 \mu\text{g Hg/m}^3$  (range  $0.0007$  to  $0.042 \mu\text{g/m}^3$ ) compared to 54 controls with no history of occupational exposure to mercury. Exposed dentists were adequately matched to the control group for age, amount of fish consumption, and number of amalgam fillings. Air concentrations were measured with personal sampling badges over typical working hours (8-10 hours/day) and converted to a TWA. Blood samples were also taken (average  $9.8 \mu\text{g/L}$ ). The average concentration in air was estimated at  $23 \mu\text{g Hg/m}^3$  when the methods of Roels *et al.* (1987) were used. The average duration in this study of dentists was shorter than the above studies, only 5.5 years. The performance of the dentists was significantly worse than controls on a number of neurobehavioral tests measuring motor speed (finger tapping), visual scanning, visuomotor coordination and concentration, visual memory, and visuomotor coordination speed. These neurobehavioral changes are consistent with central and peripheral neurotoxicity commonly observed in cases of chronic mercury toxicity.

Liang *et al.* (1993) investigated workers in a fluorescent lamp factory with a computer-administered neurobehavioral evaluation system and a mood-inventory profile. The cohort consisted of 88 individuals (19 females and 69 males) exposed for at least 2 years prior to the study. Exposure was monitored with area samplers and ranged from 8 to  $85 \mu\text{g Hg/m}^3$  across worksites. The average level of exposure was estimated at  $33 \mu\text{g Hg/m}^3$  and the average duration of exposure was estimated at 15.8 years. The exposed cohort performed significantly worse than the controls on tests of finger tapping, mental arithmetic, two digit searches, switching attention, and visual reaction time. The effect of performance persisted after controlling for chronological age as a confounding factor.

## **V. Effects of Exposure to Animals**

In laboratory animals mercury exposure resulted in adverse neurological and behavioral changes. Rabbits exposed to  $28.8 \text{ mg/m}^3$  mercury vapor for 1 to 13 weeks exhibited unspecified pathological changes, marked cellular degeneration, and necrosis in the brain (Ashe *et al.*, 1953). Rats exhibited a decline in conditioned avoidance response with exposure to  $3 \text{ mg/m}^3$  mercury vapor for 12 to 42 weeks. No histopathological changes were evident (Kishi *et al.*, 1978).

Congested lungs were observed in rats exposed to  $1 \text{ mg/m}^3$  mercury vapor for 6 weeks, 100 hours/week (Gage, 1961). Rats exposed intermittently to  $3 \text{ mg/m}^3$  mercury vapor for 12 to 42 weeks for 3 hours/day showed no changes in the respiratory system were not seen.

Rats exposed intermittently to  $2.5 \text{ mg/m}^3$  mercury vapor for 21 days demonstrated prolongation of the estrous cycle and a decrease in the number of living fetuses (Baranski and Szymczyk, 1973), however, no differences in developmental abnormalities were observed.

## VI. Derivation of Reference Exposure Levels

### *Derivation of U.S. EPA Reference Concentration (RfC)*

<i>Study</i>	Piikivi and Hanninen (1989)
<i>Study population</i>	Humans
<i>Exposure method</i>	Inhalation of workplace air
<i>Critical Effects</i>	Psychological disturbances
<i>LOAEL</i>	25 µg/m <sup>3</sup> (3 ppb)
<i>NOAEL</i>	Not observed
<i>Exposure continuity</i>	8 hours per day (10 m <sup>3</sup> /workday), 5 days/week
<i>Average experimental exposure</i>	9 µg/m <sup>3</sup> for LOAEL group
<i>Human equivalent concentration</i>	9 µg/m <sup>3</sup>
<i>Exposure duration</i>	14 year average
<i>Subchronic uncertainty factor</i>	1
<i>LOAEL uncertainty factor</i>	3
<i>Interspecies uncertainty factor</i>	1
<i>Intraspecies uncertainty factor</i>	3
<i>Modifying factor</i>	3 (lack of developmental and reproductive toxicity data)
<i>Cumulative uncertainty factor</i>	30
<i>Inhalation reference exposure level</i>	0.3 µg/m <sup>3</sup> (0.04 ppb)

This study was chosen to calculate the chronic REL because of the finding of a statistically significant increase in subjective psychological disturbances. Subjective symptoms and psychological performances were examined in 60 chloralkali workers using a computer administered test battery. Increases in memory disturbances and sleep disorders were found in exposed versus unexposed individuals, however, objective disturbance in perceptual motor, memory, or learning abilities were not found. One weakness of this study is that mercury concentrations in air were extrapolated from blood levels based on the conversion factor of Roel *et al.* (1987). A mean blood level of 10 µg/L corresponded to an average air exposure level of 25 µg Hg/m<sup>3</sup> for the group.

The human studies consistently demonstrate a LOAEL of approximately 25 µg Hg/m<sup>3</sup> in air. It is noteworthy that none of the above studies discussed in sufficient detail a dose-response relationship between mercury vapor inhalation and the toxic effects measured. Because none of the studies mention a level below which toxic effects were not seen after evaluation (a NOAEL), the extrapolation from a LOAEL to a NOAEL should be regarded with caution. Secondly, one study (Ngim *et al.*, 1992) demonstrated neurotoxic effects from mercury inhalation at an exposure level similar to the above studies, but for a much shorter duration. No adjustment was made for lifetime average exposure since one study demonstrated effects after 5 years. It is possible, however that mercury could cause neurotoxic effects after a shorter exposure period than that used in derivation of the chronic REL.

The strengths of the inhalation REL include the use of human exposure data from workers exposed over a period of years. Major areas of uncertainty are the lack of observation of a NOAEL, the limited nature of the study, the uncertainty in estimating exposure and the potential variability in exposure concentration, and the lack of reproductive and developmental toxicity studies.

***Derivation of U.S. EPA Reference Dose (RfD)***

<i>Study</i>	U.S. EPA, 1987)
<i>Study population</i>	Brown Norway rats
<i>Exposure method</i>	feeding and subcutaneous application
<i>Critical effects</i>	autoimmune effects in kidney
<i>LOAEL</i>	0.226 mg/kg-day (feeding); 0.317 mg/kg-day (subcutaneous)
<i>NOAEL</i>	none
<i>Exposure continuity</i>	
<i>Exposure duration</i>	up to 60 days
<i>Average experimental exposure</i>	
<i>LOAEL uncertainty factor</i>	10
<i>Subchronic uncertainty factor</i>	10
<i>Interspecies uncertainty factor</i>	10
<i>Intraspecies factor</i>	(The intraspecies and interspecies factors were combined into one factor of 10 to avoid an exceedingly large uncertainty factor.)
<i>Cumulative uncertainty factor</i>	1000
<i>Oral reference exposure level</i>	0.0003 mg/kg-day

Factors and Assumptions -- Dose conversions in the three studies employed a 0.739 factor for  $\text{HgCl}_2$  to  $\text{Hg}^{2+}$ , a 100% factor for subcutaneous (s.c.) to oral route of exposure, and a time-weighted average for days/week of dosing. This RfD is based on the back calculations from a Drinking Water Equivalent Level (DWEL), recommended to and subsequently adopted by the Agency, of 0.010 mg/L: ( $\text{RfD} = 0.010 \text{ mg/L} \times 2 \text{ L/day/70 kg bw} = 0.0003 \text{ mg/kg bw/day}$ ). The LOAEL exposure levels, utilized in the three studies selected as the basis of the recommended DWEL, are from Druet *et al.* (1978), Bernaudin *et al.* (1981) and Andres (1984), respectively.

The oral Reference Exposure Level for mercuric chloride is the U.S. EPA's RfD (IRIS, 1996). The principal study used was: U.S. EPA. 1987. Peer Review Workshop on Mercury Issues. Summary Report. Environmental Criteria and Assessment Office, Cincinnati, OH. October 26-27. On October 26-27, 1987, a panel of mercury experts met at a Peer Review Workshop on Mercury Issues in Cincinnati, Ohio, and reviewed outstanding issues concerning the health effects and risk assessment of inorganic mercury. The following five consensus conclusions and recommendations were agreed to as a result of this workshop: 1) The most sensitive adverse effect for mercury risk assessment is formation of mercuric-mercury-induced autoimmune glomerulonephritis. The production and deposition of IgG antibodies to the glomerular basement

membrane can be considered the first step in the formation of this mercuric-mercury-induced autoimmune glomerulonephritis. 2) The Brown Norway rat should be used for mercury risk assessment. The Brown Norway rat is a good test species for the study of  $\text{Hg}^{2+}$ -induced autoimmune glomerulonephritis. The Brown Norway rat is not unique in this regard (this effect has also been observed in rabbits). 3) The Brown Norway rat is a good surrogate for the study of mercury-induced kidney damage in sensitive humans. For this reason, the uncertainty factor used to calculate criteria and health advisories (based on risk assessments using the Brown Norway rat) should be reduced by 10-fold. 4)  $\text{Hg}^{2+}$  absorption values of 7% from the oral route and 100% from the s.c. route should be used to calculate criteria and health advisories. 5) A DWEL of 0.010 mg/L was recommended based on the weight of evidence from the studies using Brown Norway rats and limited human tissue data. Three studies using the Brown Norway rat as the test strain were chosen from a larger selection of studies as the basis for the panel's recommendation of 0.010 mg/L as the DWEL for inorganic mercury. The three studies are presented below for the sake of completeness. It must be kept in mind, however, that the recommended DWEL of 0.010 mg/L and back calculated oral RfD of 0.0003 mg/kg-day were arrived at from an intensive review and workshop discussions of the entire inorganic mercury data base, not just from one study. In the Druet *et al.* (1978) study, the duration of exposure was 8-12 weeks; s.c. injection was used instead of oral exposure. In this study the development of kidney disease was evaluated. In the first phase the rats developed anti-GBM antibodies. During the second phase, which is observed after 2-3 months, the patterns of fixation of antisera changed from linear to granular as the disease progressed. The immune response was accompanied by proteinuria and in some cases by a nephrotic syndrome. Both male and female Brown Norway rats 7-9 weeks of age were divided into groups of 6-20 animals each. The numbers of each sex were not stated. The animals received s.c. injections of mercuric chloride ( $\text{HgCl}_2$ ) 3 times weekly for 8 weeks, with doses of 0, 100, 250, 500, 1000 and 2000  $\mu\text{g/kg}$ . An additional group was injected with a 50  $\mu\text{g/kg}$  dose for 12 weeks. Antibody formation was measured by the use of kidney cryostat sections stained with a fluoresceinated sheep anti-rat IgG antiserum. Urinary protein was assessed by the biuret method (Druet *et al.*, 1978). Tubular lesions were observed at the higher dose levels. Proteinuria was reported at doses of 100  $\mu\text{g/kg}$  and above, but not at 50  $\mu\text{g/kg}$ . Proteinuria was considered a highly deleterious effect, given that affected animals developed hypoalbuminemia and many died. Fixation of IgG antiserum was detected in all groups except controls (Druet *et al.*, 1978). Bernaudin *et al.* (1981) reported that mercurials administered by inhalation or ingestion to Brown Norway rats developed a systemic autoimmune disease. The  $\text{HgCl}_2$  ingestion portion of the study involved the forcible feeding of either 0 or 3000  $\mu\text{g/kg-week}$  of  $\text{HgCl}_2$  to male and female Brown Norway rats for up to 60 days. No abnormalities were reported using standard histological techniques in either experimental or control rats. Immunofluorescence histology revealed that 80% (4/5) of the mercuric-exposed rats were observed with a linear IgG deposition in the glomeruli after 15 days of exposure. After 60 days of  $\text{HgCl}_2$  exposure, 100% (5/5) of the rats were seen with a mixed linear and granular pattern of IgG deposition in the glomeruli and granular IgG deposition in the arteries. Weak proteinuria was observed in 60% (3/5) of the rats fed  $\text{HgCl}_2$  for 60 days. The control rats were observed to have no deposition of IgG in the glomeruli or arteries as well as normal urine protein concentrations. Andres (1984) administered  $\text{HgCl}_2$  (3 mg/kg in 1 mL of water) by gavage to five Brown Norway rats and two Lewis rats twice a week for 60 days. A sixth Brown Norway rat was given only 1 mL of water by gavage twice a week for 60 days. All rats had free access to tap

water and pellet food. After 2-3 weeks of exposure, the Brown Norway HgCl<sub>2</sub>-treated rats started to lose weight and hair. Two of the HgCl<sub>2</sub>-treated Brown Norway rats died 30-40 days after beginning the study. No rats were observed to develop detectable proteinuria during the 60-day study. The kidneys appeared normal in all animals when evaluated using standard histological techniques, but examination by immunofluorescence showed deposits of IgG present in the renal glomeruli of only the mercuric-treated Brown Norway rats. The Brown Norway treated rats were also observed with mercury-induced morphological lesions of the ileum and colon with abnormal deposits of IgA in the basement membranes of the intestinal glands and of IgG in the basement membranes of the lamina propria. All observations in the Lewis rats and the control Brown Norway rat appeared normal.

The U.S. EPA reported its confidence in the RfD as: Data Base - High and RfD - High. No one study was found adequate for deriving an oral RfD; however, based on the weight of evidence from the studies using Brown Norway rats and the entirety of the mercuric mercury data base, an oral RfD of high confidence was derived.

## VII. References

Albers JW, Kallenbach LR, Fine LJ, Wolfe RA, Donofrio PD, Alessi AG, Stolp-Smith KA, and Bromberg MB, Mercury Workers Study Group. 1988. Neurological abnormalities associated with remote occupational elemental mercury exposure. *Annals of Neurology* 24:651-659.

ACGIH. 1991. American Conference of Governmental Industrial Hygienists. Documentation of the Threshold Limit Values and Biological Exposure Indices. 6th edition. Cincinnati, OH, ACGIH.

Ashe WF, Largent EJ, Dutra FR, Hubbard DM, and Blackstone M. 1953. Behavior of mercury in the animal organism following inhalation. *AMA Archives of Industrial Hygiene and Occupational Medicine* 7:19-43.

Baranski B, and Szymezyk I. 1973. Effects of mercury vapor upon reproductive function of the female white rat. *Medical Practice* 24:249-261.

Berregard L, Hultberg B, Schultz A, and Sallsten G. 1988. Enzymuria in workers exposed to inorganic mercury. *International Archives of Occupational and Environmental Health* 61:65-69.

Bernard AM, Roels HR, Foidart JM, and Lauwerys RL. 1987. Search for anti-laminin antibodies in the serum of workers exposed to cadmium, mercury vapour, or lead. *International Archives of Occupational and Environmental Health* 59:303-309.

Chaffin DB, Dinman BD, Miller JM, Smith RG, and Zontine DH. 1973. An evaluation of the effects of chronic mercury exposures on EMG and psychomotor functions. Washington DC: U.S. Department of Health, Education, and Welfare, National Institute for Occupational Safety and Health; contract no. HSM-099-71-62.

Fagala GE, and Wigg CL. 1992. Psychiatric manifestations of mercury poisoning. *Journal of American Academy of Child Adolescent Psychiatry* 31:306-311.

Fawer RF, De Reibaupierre Y, Guillemin MP, Berode M, and Lob M. 1983. Measurement of hand tremor induced by industrial exposure to metallic mercury. *British Journal of Industrial Medicine* 40:204-208.

Gage. 1961. The distribution and excretion of inhaled mercury vapour. *British Journal of Industrial Medicine* 18:287-294.

HSDB. 1994. Hazardous Substances Data Bank, Micromedex, Denver, CO.

Kishi R, Hashimoto K, Shimizu S, Kobayashi M. 1978. Behavioral changes and mercury concentrations in tissues of rats exposed to mercury vapor. *Toxicology and Applied Pharmacology* 46:555-566.

Kishi R, Doi R, Fukuchi Y, Satoh H, Satoh T, Ono A, Moriwaka F, Tashiro K, Takahara N, Sasatani H, Shirakashi H, Kamada T, and Nakagawa K. 1993. Subjective symptoms and neurobehavioral performances of ex-mercury miners at an average of 18 years after the cessation of chronic exposure to mercury vapor. *Environmental Research* 62:289-302.

Langolf GD, Chaffin DB, Henderson R, and Whittle HP. 1978. Evaluation of workers exposed to elemental mercury using quantitative tests of tremor and neuromuscular functions. *American Industrial Hygiene Association Journal* 39:976-985.

Liang Y-X, Sun R-K, Chen Z-Q, and Li L-H. 1993. Psychological effects of low exposure to mercury vapor: Application of a computer-administered neurobehavioral evaluation system. *Environmental Research* 60:320-327.

Ngim CH, Foo SC, Boey KW, and Jeyaratnam J. 1992. Chronic neurobehavioral effects of elemental mercury in dentists. *British Journal of Industrial Medicine* 49:782-790.

Piikivi L. 1989. Cardiovascular reflexes and low long-term exposure to mercury vapour. *International Archives of Occupational and Environmental Health*. 61:391-395.

Piikivi L, and Hanninen H. 1989. Subjective symptoms and psychological performance of chlorine-alkali workers. *Scandinavian Journal of Work Environment and Health* 15:69-74.

Piikivi L, and Ruokonen A. 1989. Renal function and long-term low mercury vapor exposure. *Archives of Environmental Health* 44:146-149.

Piikivi L, and Tolonen U. 1989. EEG findings in chlor-alkali workers subjected to low long term exposure to mercury vapour. *British Journal of Industrial Medicine* 46:370-375.

Piikivi L, Hanninen H, Martelin T, and Mantere P. 1984. Psychological performance and long-term exposure to mercury vapors. *Scandinavian Journal of Work Environment and Health* 10:35-41.

Roels H, Lauwerys R, Buchet JP, Bernard A, Barthels A, Oversteins M, and Gaussin J. 1982. Comparison of renal function and psychomotor performance in workers exposed to elemental mercury. *International Archives of Occupational and Environmental Health* 50:77-93.

Smith RG, Vorwald AJ, Patil LS, and Mooney TF Jr. 1970. Effects of exposure to mercury in the manufacture of chlorine. *American Industrial Hygiene Association Journal* 31:687-700.

Taug C, Sanfilipo DJ, Rowens B, Szejda J, and Hesse JL. 1992. Acute and chronic poisoning from residential exposures to elemental mercury-Michigan, 1989-1990. *Journal of Toxicology and Clinical Toxicology* 30:63-67.

U.S. EPA. 1995. United States Environmental Protection Agency. Integrated Risk Information System (IRIS) database. Documentation of the reference concentration for chronic inhalation exposure (RfC) for elemental mercury.

CHRONIC TOXICITY SUMMARY

**METHANOL**

(methyl alcohol, wood spirit, carbinol, wood alcohol, wood naphtha)

**CAS Registry Number: 67-56-1**

**I. Chronic Toxicity Exposure Level**

<i>Inhalation reference exposure level</i>	<b>10 mg/m<sup>3</sup></b>
<i>Critical effect(s)</i>	Increased incidence of abnormal cervical ribs, cleft palate, and exencephaly in mice
<i>Hazard index target(s)</i>	Teratogenicity

**II. Chemical Property Summary (HSDB, 1995)**

<i>Molecular formula</i>	CH <sub>3</sub> OH
<i>Molecular Weight</i>	32.04 g/mol
<i>Vapor Pressure</i>	92 torr at 20°C
<i>Solubility</i>	Methanol is miscible with water, ethanol, ether and many other organic solvents
<i>Color</i>	Colorless
<i>Conversion Factor</i>	1 ppm = 1.31 mg/m <sup>3</sup>

**III. Major Uses and Sources**

Originally distilled from wood, methanol is now manufactured synthetically from carbon oxides and hydrogen. Methanol is used primarily for the manufacture of other chemical and as a solvent. It is also added to a variety of commercial and consumer products such as windshield washing fluid and de-icing solution, duplicating fluids, solid canned fuels, paint remover, model airplane fuels, embalming fluids, lacquers, and inks. Methanol is also used as an alternative motor fuel.

**IV. Effects of Human Exposure**

The majority of the available information on methanol toxicity in humans relates to acute rather than chronic exposure. The toxic effects after repeated or prolonged exposure to methanol are believed to be qualitatively similar but less severe than those induced by acute exposure (Kavet and Nauss, 1990). These effects include CNS and visual disturbances such as headaches, dizziness, nausea and blurred vision. The role of formate, a metabolite of methanol, in chronic

toxicity is unclear. In one study, symptoms of blurred vision, headaches, dizziness, nausea and skin problems were reported in teachers aides exposed to duplicating fluid containing 99% methanol (Frederick *et al.*, 1984). Individual aides worked as little as 1 hr/day for 1 day a week to 8 hrs/day for 5 days/wk. The workers' total exposure duration was not mentioned. A dose-response relationship was observed between the self-reported amount of time spent at the duplicator and the incidence of symptoms. The concentrations of methanol in the breathing zones near the machines in 12 schools ranged from 485 to 4096 mg/m<sup>3</sup> (365 to 3080 ppm) for a 15 minute sample.

Forty-five percent of duplicating machine operators experienced blurred vision, headache, nausea, dizziness and eye irritation (NIOSH, 1981). Air concentrations of methanol for 25 minutes near the machines averaged 1330 mg/m<sup>3</sup>.

Employees working in the proximity of direct process duplicating machines complained of frequent headaches and dizziness (Kingsley and Hirsch, 1954). Air concentrations of methanol ranged from 15 ppm (20 mg/m<sup>3</sup>) to 375 ppm (490 mg/m<sup>3</sup>).

All of 30 young women who had polished wood pencils with a varnish containing methanol experienced headaches, gastric disorders, vertigo, nausea and blurred vision (Tyson, 1912; as cited in NIOSH, 1976).

None of the above studies specified the workers' total duration of exposure.

Ubaydullayev (1968) exposed 3 to 6 subjects to methanol vapor for short durations (40 minutes for some subjects and others for an unspecified amount of time). Electrical brain cortex reflex activity was significantly altered upon exposures to 1.17 mg/m<sup>3</sup> (0.89 ppm) or 1.46 mg/m<sup>3</sup> (1.11 ppm). No effect was observed at 1.01 mg/m<sup>3</sup> (0.77 ppm).

## **V. Effects of Animal Exposure**

With the exception of non-human primates, the signs of methanol toxicity in commonly used laboratory animals are quite different from those signs observed in humans (Gilger and Potts, 1955). The major effect of methanol in non-primates (rodents, dogs, cats, etc) is CNS depression similar to that produced by other alcohols. Metabolic acidosis and ocular toxicity are not observed. The differences in toxicity are attributed to the ability of non-primates to more efficiently metabolize formate than humans and other primates (Tephly, 1991).

Two chronic studies have been conducted with monkeys. In one study, ultrastructural abnormalities of hepatocytes indicating alteration of RNA metabolism were observed in rhesus monkeys given oral doses of 3 to 6 mg/kg methanol for 3 to 20 weeks (Garcia and VanZandt, 1969; as cited in Rowe and McCollister, 1978). In a study aimed at examining ocular effects, cynomolgus monkeys were exposed by inhalation to methanol concentrations ranging from 680 mg/m<sup>3</sup> (520 ppm) to 6650 mg/m<sup>3</sup> (5010 ppm) for 6 hours per day, 5 days per week for 4

weeks (Andrews *et al.*, 1987). No deaths occurred and no treatment-related effects were found upon histopathologic examination.

Exposure to a mixture of methanol and other solvents has been associated with central nervous system birth defects in humans (Holmberg, 1979). However, because of mixed or inadequate exposure data, methanol is not considered a known human teratogen.

In two separate studies in male rats, inhalation exposure to methanol ranging from 260 to 13,000 mg/m<sup>3</sup> for 6 to 8 hours per day for either 1 day or 1, 2, 4 or 6 weeks resulted in a significant reduction in testosterone levels (Cameron *et al.*, 1984; Cameron *et al.*, 1985).

Ubaydullayev (1968) exposed rats (15 per group) to 0, 0.57, or 5.31 mg/m<sup>3</sup> methanol continuously for 90 days. Chronaxy ratios of flexor and extensor muscles were measured in addition to hematologic parameters and acetyl cholinesterase activity. No changes were apparent in the 0.57 mg/m<sup>3</sup> group. Effects observed in the 5.31 mg/m<sup>3</sup> group included decreased blood albumin content beginning 7 weeks after exposure, slightly decreased acetylcholinesterase activity, decreased coproporphyrin levels in the urine after 7 weeks, and changes in muscle chronaxy.

Pregnant rats were exposed by inhalation to methanol at concentration ranging from 5000 to 20,000 ppm for 7 hours per day on days 1-19 gestation, and days 7-15 for the highest dose group (Nelson *et al.*, 1985). A dose-related decrease in fetal weight, an increase in extra or rudimentary cervical ribs, and urinary or cardiovascular defects were observed. Exencephaly and encephalocele were observed in the 20,000 ppm dose group. The no observed adverse effect level (NOAEL) was 5000 ppm.

Pregnant mice were exposed to methanol vapors at concentrations ranging from 1000 to 15,000 ppm for 7 hours per day on days 6-15 of gestation (Rogers *et al.*, 1993). Increased embryonic and fetal death, including an increase in full-litter resorptions, was observed at 7500 ppm and higher. Significant increases in the incidence of exencephaly and cleft palate were observed at 5000 ppm and higher. A dose-related increase in the number of fetuses per litter with cervical ribs (usually small ossification sites lateral to the seventh cervical vertebra) was observed at 2000 ppm and above. The NOAEL was 1000 ppm.

## VI. Derivation of Chronic Reference Exposure Level (REL)

<i>Study</i>	Rogers <i>et al.</i> (1993)
<i>Study population</i>	Pregnant mice
<i>Exposure method</i>	Discontinuous inhalation, 7 hours/day on days 6-15 of gestation
<i>Critical effects</i>	Abnormal cervical ribs, exencephaly, cleft palate
<i>LOAEL</i>	5000 ppm
<i>NOAEL</i>	1000 ppm
<i>Exposure continuity</i>	7 hr/day
<i>Exposure duration</i>	10 days
<i>Average experimental exposure</i>	292 ppm for NOAEL group
<i>Human equivalent concentration</i>	292 ppm for NOAEL group (gas with systemic effects, based on RGDR = 1.0 using default assumption that $\lambda(a) = \lambda(h)$ )
<i>Subchronic uncertainty factor</i>	1 (see below)
<i>LOAEL uncertainty factor</i>	1
<i>Interspecies uncertainty factor</i>	3
<i>Intraspecies uncertainty factor</i>	10
<i>Cumulative uncertainty factor</i>	30
<i>Inhalation reference exposure level</i>	10 ppm (10,000 ppb, 10 mg/m <sup>3</sup> , 10,000 µg/m <sup>3</sup> )

A NOAEL of 1000 ppm for developmental malformations was observed in mice exposed for 7 hours/day on days 6 through 15 of gestation (Rogers *et al.*, 1993). Although not a chronic study, the endpoint, teratogenicity, is a function of exposure only during gestation, especially in the case of a non-accumulating compound such as methanol. Therefore, an uncertainty factor for subchronic to chronic was not required. The investigators calculated maximum likelihood estimates (MLEs) using a log-logistic model for both 1% and 5% added risks above background. The most sensitive developmental toxicity endpoint was an increase in the incidence of cervical ribs. The MLE<sub>05</sub> and BMC<sub>05</sub> for cervical ribs were 824 ppm (1079 mg/m<sup>3</sup>) and 305 ppm (400 mg/m<sup>3</sup>), respectively.

Andrews *et al.* (1987) have investigated the effects of chronic exposure to methanol in primates. In this study aimed at examining ocular toxicity, no treatment-related effects were observed in cynomolgus monkeys exposed by inhalation to 6650 mg/m<sup>3</sup> (5010 ppm) methanol for 6 hours/day, 5 days/week for 4 weeks. However, Andrews *et al.* did not examine possible neurologic or reproductive effects which have been observed in other species at lower concentrations (see Sections IV and V). Teachers aides exposed to duplicating fluid containing 99% methanol reported symptoms of blurred vision, headaches, dizziness, nausea and skin problems (Frederick *et al.*, 1984). The measured methanol concentrations ranged from 485 to 4096 mg/m<sup>3</sup> for a 15-minute sampling period. Exposure assessment in this study was poorly characterized and exposure duration was not specified.

The major strengths of the REL are the observation of a NOAEL and the demonstration of a dose-response relationship. The major uncertainties are the lack of human data for chronic inhalation exposure, the lack of comprehensive, long-term multiple dose studies, and the difficulty in addressing reproductive short-term effects within the chronic REL framework.

## VIII. References

- Andrews LS, Clary JJ, Terrill JB, and Bolte HF. 1987. Subchronic inhalation toxicity of methanol. *J Toxicol Environ Health*, 20:117-24.
- Cameron AM, Nilsen OG, Haug E, and Eik-Nes KB. 1984. Circulating concentrations of testosterone, luteinizing hormone and follicle stimulating hormone in male rats after inhalation of methanol. *Arch Toxicol Suppl*, 7.
- Cameron AM, Zahlse K, Haug E, Nilsen OG, and Eik-Nes KB. 1985. Circulating steroids in male rats following inhalation of n-alcohols. *Arch Toxicol Suppl*, 8:422 [as cited in Kavet and Nauss, 1990].
- Frederick LJ, Schlulte PA, and Apol A. 1984. Investigation and control of occupational hazards associated with the use of spirit duplicators. *Am Ind Hyg Assoc J*, 45:51-5.
- Garcia JH, and VanZandt JP. 1969. *Proc Electron Microsc Soc Am*, 27:360 [as cited in Rowe and McCollister, 1978].
- Gilger AP, and Potts AM. 1955. Studies on the visual toxicity of methanol: V. The role of acidosis in experimental methanol poisoning. *Am J Ophthalmol*, 39:63-86.
- Holmberg PC. 1979. Central nervous system defects in children born to mothers exposed to organic solvents during pregnancy. *Lancet*, 2:177-9.
- HSDB. 1995. Hazardous Substances Data Bank. National Library of Medicine, Bethesda, Maryland (CD-ROM Version). Micromedex, Inc., Denver, Colorado (Edition expires 7/31/96).
- Kavet R, and Nauss KM. 1990. The toxicity of inhaled methanol vapors. *Crit Rev Toxicol*, 21:21-50.
- Kingsley WH, and Hirsch FG. 1954. Toxicologic considerations in direct process spirit duplicating machines. *Comp Med*, 6:7-8.
- Nelson BK, Brightwell WS, MacKenzie DR, Khan A, Burg JR, Weigel WW, and Goad PT. 1985. Teratological assessment of methanol and ethanol at high inhalation levels in rats. *Fundam Appl Toxicol*, 5:727-36.

NIOSH. 1976. National Institute for Occupational Safety and Health. Criteria document for methyl alcohol. Cincinnati, OH.

NIOSH. 1981. National Institute for Occupational Safety and Health. Health Hazard Evaluation Report, HETA 81-177, PB82-194648.178-88 [as cited in Kavet and Nauss, 1990].

Rogers JM, Mole ML, Chernoff N, Barbee BD, Turner CI, Logsdon TR, and Kavlock JV. 1993. The developmental toxicity of inhaled methanol in the CD-1 mouse, with quantitative dose-response modeling for estimation of benchmark doses. *Teratology*, 47:175-88.

Rowe RK, and McCollister SB. 1978. Alcohols. In: Patty's Industrial Hygiene and Toxicology. Clayton and Clayton (eds). pp.4527-41.

Tephly. 1991. Minireview. The toxicity of methanol. *Life Sci*, 48:1031-41.

Tyson HH. 1912. Amblyopia from inhalation of methyl alcohol. *Arch Ophthalmol*, 16:459-71 [as cited in NIOSH, 1976].

Ubaydullayev R. 1968. A study of hygienic properties of methanol as an atmospheric air pollutant (translation by B.S. Levine). *USSR Literature on Air Pollution and Related Occupational Diseases - A Survey*. 17:39-45.

CHRONIC TOXICITY SUMMARY

**METHYL BROMIDE**

(bromomethane; monobromomethane)

**CAS Registry Number: 74-83-9**

**I. Chronic Toxicity Summary**

<i>Inhalation reference exposure level</i>	<b>5 <math>\mu\text{g}/\text{m}^3</math></b> (U.S. EPA RfC) This document summarizes the evaluation of non-cancer health effects by U.S. EPA for the RfC
<i>Critical effect(s)</i>	Histological lesions of the olfactory epithelium of the nasal cavity in rats
<i>Hazard index target(s)</i>	Respiratory system; nervous system; teratogenicity

**II. Physical and Chemical Properties (HSDB 1994)**

<i>Molecular formula</i>	CH <sub>3</sub> Br
<i>Molecular weight</i>	94.95 g/mol
<i>Description</i>	Colorless gas
<i>Specific gravity</i>	1.73 @ 0°C
<i>Boiling point</i>	3.6°C
<i>Vapor pressure</i>	1420 mm Hg @ 20°C
<i>Solubility</i>	Soluble in ethanol, benzene, carbon disulfide, and 1.75% (w/w) in water
<i>Odor threshold</i>	20.6 ppm
<i>Odor description</i>	Sweetish odor
<i>Metabolites</i>	Methanol, bromide, 5-methylcysteine
<i>Conversion factor</i>	1 ppm = 3.89 mg/m <sup>3</sup> @ 25° C

**III. Major Uses and Sources**

Methyl bromide (MeBr) was used historically as an industrial fire extinguishing agent introduced in the U.S. from Europe in the 1920s. Current uses of MeBr include the fumigation of homes and other structures for termites and other pests. Methyl bromide is also used to fumigate soil before planting and fruits and vegetables after harvest. In 1981, 6.3 million pounds of MeBr were reportedly used in California (Alexeeff and Kilgore, 1983). By 1991, its use had grown to 18.7 million pounds in the state (Cal/EPA, 1993).

#### IV. Effects of Human Exposure

Workers (n = 32) exposed to MeBr during fumigation of soil or structures were compared to a referent group of 29 workers not exposed to MeBr, but exposed to other fumigants (Anger *et al.*, 1986). Exposures to MeBr were not quantified. It was found that workers exposed to MeBr had a higher rate of neurological symptoms and performed less well on several behavioral tests. Several confounding factors were present in this study, including lack of adjustments for age, alcohol consumption, prescription medication, illegal drugs, education, or ethnic group between the exposed and the referent groups.

#### V. Effects of Animal Exposure

The first experimental animal study on repeated MeBr exposures was carried out and reported by Irish and associates (1940). In this study, rats (135 per group), rabbits (104 per group), or female rhesus monkeys (13 per group) were exposed to 0, 17, 33, 66, 100, or 220 ppm (0, 66, 128, 256, 388, or 853 mg/m<sup>3</sup>) 7-8 hours/day, 5 days/week for 6 months or until the majority of the animals exhibited severe signs of toxicity. Mortality was seen in rats, guinea pigs, and monkeys at 100 ppm. Rabbits began to die at 33 ppm. Severe effects, including paralysis, were seen after exposure to 66 ppm in rabbits and monkeys. None of the species exhibited adverse effects after exposure to 17 ppm.

Kato and associates (1986) observed focal lesions in the brain and heart in rats (10-12 per group) after inhalation of 150 ppm (585 mg/m<sup>3</sup>) MeBr 4 hours/day, 5 days/week for 11 weeks. In another experiment, rats were exposed to 0, 200, 300, or 400 ppm (0, 777, 1160, or 1550 mg/m<sup>3</sup>) MeBr 4 hours/day, 5 days/week for 6 weeks. In this experiment, rats exposed to any concentration of MeBr exhibited coronary lesions, and exposures of 300 ppm or greater resulted in neurological dysfunction, including ataxia and paralysis. Testicular atrophy was noted in 6 of the 8 animals exposed to 400 ppm.

Anger *et al.* (1981) determined that rabbits are more sensitive than rats to neurotoxicity of MeBr. In this study, rats or rabbits were exposed to 0 or 65 ppm (0 or 254 mg/m<sup>3</sup>) MeBr for 7.5 hours/day, 4 days/week, for 4 weeks. Nerve conduction velocity and eyeblink reflex were impaired in the rabbits but not rats exposed to 65 ppm MeBr. Similarly, rats did not exhibit neurological signs after exposure to 55 ppm (215 mg/m<sup>3</sup>) MeBr for 36 weeks. Rabbits exposed to 26.6 ppm (104 mg/m<sup>3</sup>) did not display any neurological effects after 8 months exposure (Russo *et al.*, 1984).

In the studies of Reuzel and associates (1987, 1991), groups of 50 male and 60 female Wistar rats were exposed to 0, 3, 30, or 90 ppm methyl bromide (98.8%) for 6 hours per day and 5 days per week. Three groups of animals (10/sex/exposure level) were killed for observations at 14, 53, and 105 weeks of exposure. Body weight, hematology, clinical chemistry, and urinalyses were examined throughout the experiment in addition to histopathology and organ weights at time of necropsy. Exposures of males and females to 90 ppm resulted in reduced body weight. Exposure to 90 ppm also resulted in significant lesions in the heart in the form of cartilaginous

metaplasia and thrombus in the males, and myocardial degeneration and thrombus in the females. Exposure of males to 30 or 90 ppm resulted in a decrease in relative kidney weight. Histological changes in the nose, heart, esophagus, and forestomach were the principal effects of methyl bromide toxicity. At the lowest concentration (3 ppm), very slight degenerative changes in the nasal epithelium, and olfactory basal cell hyperplasia were noted in both sexes at 29 months. Based on this study, a LOAEL of 3 ppm (11.7 mg/m<sup>3</sup>) was determined.

The National Toxicology Program (NTP) conducted a 13-week and a chronic study on the toxicology and carcinogenesis of methyl bromide in rats and mice (NTP, 1990). In the 13-week study, 18 rats/sex/group were exposed to 0, 30, 60, or 120 ppm (0, 117, 233, or 466 mg/m<sup>3</sup>) MeBr 6 hours/day, 5 days/week. The mice were exposed to 0, 10, 20, 40, 80, or 120 ppm (0, 39, 78, 155, 311, or 466 mg/m<sup>3</sup>) 6 hours/day, 5 days/week. Hematological parameters and selected organ weights were measured in both species, in addition to histopathological changes. Pseudocholinesterase activity and neurobehavioral tests were conducted in the mice. Serious effects, including 58% body weight loss, 17% mortality and severe curling and crossing of the hindlimbs were observed in mice exposed to 120 ppm MeBr. Exposure of males to 40 ppm or higher resulted in significant effects on several hematological parameters, including decreased mean cell hemoglobin and increased red blood cell count. The only exposure-related histological effect was olfactory epithelial dysplasia and cysts in the rats of both sexes exposed to 120 ppm.

A 6-week study in rats and mice (5 animals/sex/group) exposed to 0 or 160 ppm (0 or 624 mg/m<sup>3</sup>) showed high mortality rates, loss in body weight and histological changes in multiple organ systems including brain, kidney, nasal cavity, heart, adrenal gland, liver, and testes (NTP, 1990).

An exposure of mice (86 animals/group) to 0, 10, 33, or 100 ppm (0, 38.8, 128, or 388 mg/m<sup>3</sup>) MeBr for 6 hours/day, 5 days/week, for 103 weeks was also conducted by NTP (1990). In this study, high mortality rates in both males and females in the 100 ppm group resulted in a discontinuation of exposure after 20 weeks. A low incidence of sternal dysplasia and a significant decrease in locomotor activity were noted in the 10 ppm group.

A 5-day exposure of rats (10 animals/group) to 0, 90, 175, 250, or 325 ppm (0, 350, 680, 971, or 1260 mg/m<sup>3</sup>) resulted in lesions in the nasal olfactory sensory cells, the cerebellum and adrenal gland beginning at 175 ppm (Hurt et al., 1987). Hurt and Working (1988) later observed severe histological damage to the nasal epithelium following a single exposure to 90 or 200 ppm (351 or 780 mg/m<sup>3</sup>) MeBr. Olfactory function, measured by the ability to locate buried food, was impaired at the 200 ppm exposure. In this study, reduced testosterone and testicular glutathione levels were observed in the male rats exposed to 200 ppm, but no effects on spermatogenesis, sperm quality, or testes histopathology were noted.

Sikov et al. (1981) examined the teratogenic potential of MeBr in rats and rabbits exposed to 0, 20, or 70 ppm (0, 78, or 272 mg/m<sup>3</sup>) 7 hours/day, 5 days/week for 3 weeks during days 1-19 (rats) or 1-24 (rabbits) of gestation. No maternal or fetal effects were observed in the rats, however, severe maternal neurotoxic effects were observed in the rabbits that resulted in 24/25

deaths. In this study, no significant maternal or fetal effects were observed at a concentration of 20 ppm.

Another developmental toxicity study was conducted in rabbits by Breslin *et al.* (1990). In this study, rabbits were exposed to 0, 20, 40, or 80 ppm (0, 78, 156, or 312 mg/m<sup>3</sup>) MeBr for 6 hours/day on gestation days 6-19. Maternal toxicity was observed at 80 ppm and included reduced body weight gain and signs of neurotoxicity. In addition to the maternal effects observed, a significant increase in incidence of gall bladder agenesis and fused sternebrae were observed in the offspring exposed to 80 ppm. No adverse effects were observed at 40 ppm or lower concentrations.

A 2-generation reproduction and developmental toxicity study on MeBr in rats was conducted by American Biogenics Corporation (1986). In this study, groups of rats (25/sex/concentration) were exposed to 0, 3, 30, or 90 ppm (0, 12, 117, or 350 mg/m<sup>3</sup>) MeBr 6 hours/day, 5 days/week during premating, gestation, and lactation through 2 generations. Significant decreases in body weight during the pre-mating period and at the end of the study were observed in the males exposed to 90 ppm. Although some adult organ weights were affected in the 90-ppm group, there was no evidence of histopathology in these organs. Neonatal body weights were decreased by exposure to 30 ppm. There was a decreased cerebral cortex width of the 90 ppm F<sub>1</sub> group, reduced brain weight of 30 ppm F<sub>1</sub> females, and reduced fertility of 30 and 90 ppm F<sub>2b</sub> groups.

## VI. Derivation of U.S. EPA RfC

<i>Study</i>	Reuzel <i>et al.</i> , 1987; 1991; U.S. EPA, 1995
<i>Study population</i>	Male and female Wistar rats (50 and 60 per group, respectively)
<i>Exposure method</i>	Discontinuous inhalation exposures (0, 3, 30, or 90 ppm) over 29 months
<i>Critical effects</i>	Histological lesions of the olfactory epithelium of the nasal cavity
<i>LOAEL</i>	3 ppm
<i>NOAEL</i>	Not observed
<i>Exposure continuity</i>	6 hr/day, 5 days/week
<i>Exposure duration</i>	29 months
<i>Average experimental exposure</i>	0.54 ppm for the LOAEL group
<i>Human equivalent concentration</i>	0.12 ppm for the LOAEL group (gas with extrathoracic respiratory effects, RGDR = 0.23, based on MV = 0.03 m <sup>3</sup> /min, SA = 11.6 cm <sup>2</sup> )
<i>LOAEL uncertainty factor</i>	3
<i>Subchronic uncertainty factor</i>	1
<i>Interspecies uncertainty factor</i>	3
<i>Intraspecies uncertainty factor</i>	10
<i>Cumulative uncertainty factor</i>	100
<i>Inhalation reference exposure level</i>	0.001 ppm (1 ppb, 0.005 mg/m <sup>3</sup> , 5 µg/m <sup>3</sup> )

The major strengths of the REL are the use of a comprehensive, long-term multiple dose study with large sample sizes, and the availability of supporting data including long-term studies in other species and reproductive and developmental studies. The major uncertainties are the lack of human data and the lack of a NOAEL observation for the critical effect.

The California Department of Pesticide Regulation used a different approach that adjusts for respiration rate differences between humans and animals and which uses 10-fold uncertainty factors for interspecies differences, for intraspecies variability, and for a LOAEL to NOAEL extrapolation. Applying these factors to the same 3 ppm LOAEL results in a level for children and adults of 1 and 2 ppb, respectively.

## VI. References

Alexeeff GV, and Kilgore WW. 1983. Methyl bromide, in: Residue Reviews, Vol. 88. Springer-Verlag, New York., pp. 102-153.

American Biogenics Corporation. 1986. Two-generation reproduction study via inhalation in albino rats using methyl bromide. Final Report. American Biogenics Corporation Study 450-1525, OTS 0515364 sponsored by the Methyl Bromide Panel.

Anger WK, Moody L, Burg J, Brightwell WS, Taylor BJ, Russo JM, *et al.* 1986. Neurobehavioral evaluation of soil and structural fumigators using methyl bromide and sulfuryl fluoride. *Neurotoxicology*. 7(3):137-156.

Anger WK, Setzer JV, Russo JM, Brightwell WS, Wait RG, and Johnson BL. 1981. Neurobehavioral effects of methyl bromide inhalation exposures. *Scand. J. Work Environ. Health*. 7(Suppl. 4):40-47.

Breslin WJ, Zablotny CL, Brabley GJ, and Lomax LG. 1990. Methyl bromide inhalation teratology study in New Zealand white rabbits. Toxicology Research Laboratory, Health and Environmental Sciences, The Dow Chemical Company, Midland, MI. Study No. K-000681-033. OTS Number 8EHQ-1189-0844 S.

Cal/EPA. May, 1993. California Environmental Protection Agency. Letter to Carol Browner, Administrator, U.S.EPA, on methyl bromide uses in California.

HSDB. 1994. Hazardous Substance Data Bank. National Library of Medicine, Bethesda, MD (CD-ROM version). Micromedex, Inc., Denver, CO (edition expires 11/31/94).

Hurt ME, Morgan KT, and Working PK. 1987. Histopathology of acute toxic responses in selected tissues from rats exposed by inhalation to methyl bromide. *Fundam. Appl. Toxicol.* 9:352-365.

Hurt ME, and Working PK. 1988. Evaluation of spermatogenesis and sperm quality in the rat following acute inhalation exposure to methyl bromide. *Fundam. Appl. Toxicol.* 10(3):490-498.

Irish DD, Adams EM, Spencer HC, and Rowe VK. 1940. The response attending exposure of laboratory animals to vapors of methyl bromide. *J. Ind. Hyg. Toxicol.* 22(6):218-230.

Kato N, Morinobu S, and Ishizu S. 1986. Subacute inhalation experiment for methyl bromide in rats. *Indust. Health.* 24:87-103.

NTP. 1990. National Toxicology Program. Toxicology and carcinogenesis studies of methyl bromide (CAS No. 74-83-9) in B6C3F1 mice (inhalation studies). NTP TR 385, NIH Publication No. 91-2840.

Reuzel PGJ, Kuper CF, Dreef-van der Meulen HC, and Hollanders VMH. 1987. Chronic (29-month) inhalation toxicity and carcinogenicity study of methyl bromide in rats. Report No. V86.469/221044. Netherlands Organisation for Applied Scientific Research, Division for Nutrition and Food research, TNO. EPA/OTS Document No. 86-8700001202.

Reuzel PGJ, Dreef-van der Meulen HC, Hollanders VMH, Kuper CF, Feron VJ, and Van der Heijden CA. 1991. Chronic inhalation toxicity and carcinogenicity study of methyl bromide in Wistar rats. *Fd. Chem. Toxic.* 29(1):31-39.

Russo JM, Anger WK, Setzer JV, and Brightwell WS. 1984. Neurobehavioral assessment of chronic low-level methyl bromide exposure in the rabbit. *J. Toxicol. Environ. Health.* 14:247-255.

Sikov MR, Cannon WC, Carr DB, Miller RA, Montgomery LR, and Phelps DW. 1981. Teratologic assessment of butylene oxide, styrene oxide and methyl bromide. Battelle Pacific Northwest Laboratory, Richland, WA, for the National Institute for Occupational Safety and Health, Cincinnati, OH.

U.S.EPA. 1995. Environmental Protection Agency. Integrated Risk Information System (IRIS) database. Reference concentration (RfC) for methyl bromide.

CHRONIC TOXICITY SUMMARY

# METHYL CHLOROFORM

(1,1,1-Trichloroethane, methyltrichloromethane)

CAS Registry Number: 71-55-6

## I. Chronic Toxicity Summary

<i>Inhalation reference exposure level</i>	<b>1,000 µg/m<sup>3</sup></b>
<i>Critical effect(s)</i>	Astrogliosis in the sensorimotor cortex (brain) of gerbils
<i>Hazard index target(s)</i>	Nervous system

## II. Chemical Property Summary (HSDB, 1995)

<i>Molecular formula</i>	C <sub>2</sub> H <sub>3</sub> Cl <sub>3</sub>
<i>Molecular weight</i>	133.42 g/mol
<i>Description</i>	Colorless liquid
<i>Specific gravity</i>	1.3376 @ 20° C
<i>Boiling point</i>	74.1° C
<i>Melting point</i>	-30.4° C
<i>Vapor pressure</i>	127 torr @ 25° C
<i>Solubility</i>	Soluble in acetone, benzene, methanol, carbon tetrachloride
<i>Conversion factor</i>	5.47 µg/m <sup>3</sup> per ppb at 25°C

## III. Major Uses and Sources

Methyl chloroform is used as a solvent for adhesives and for metal degreasing (ACGIH, 1992). It is also used in the manufacture of vinylidene chloride and in textile processing and dry cleaning.

## IV. Effects of Human Exposure

A 44-year old woman was diagnosed with peripheral neuropathy following 18 months of occupational exposure to methyl chloroform in a solvent bath (House *et al.*, 1994). There was no identified exposure to agents known to cause peripheral neuropathy, such as n-hexane or trichloroethylene. The worker reported that she wore protective gloves and a respirator, both of

which frequently leaked. Seven months following removal from exposure, the worker showed improved nerve conduction.

Other case reports have identified the nervous system as a target of methyl chloroform toxicity in similar exposure scenarios. Three workers developed distal sensory neuropathy after working with methyl chloroform in a decreasing operation with repeated dermal exposure (Liss, 1988; Howse *et al.*, 1989). Changes were observed in nerve conduction in the upper extremities accompanied by both axonopathy and myelopathy.

Cardiac arrhythmia resulting from heightened cardiac sensitivity to epinephrine has been reported in several case reports of high acute inhalation exposures to methyl chloroform (ATSDR, 1990). There are case reports of arrhythmias persisting for two weeks or more after cessation of exposure to methyl chloroform (McLeod *et al.*, 1987).

An epidemiological study of workers chronically exposed to low levels of methyl chloroform (<250 ppm) found no changes in blood pressure, heart rate, or electrocardiogram (Kramer *et al.*, 1978). This study consisted of 151 workers who had been exposed for more than one year. No neurophysiological testing was done.

Another study of 22 female workers exposed to methyl chloroform (plus 7 unexposed control workers) at concentrations ranging from 110-345 ppm in air for a mean of 6.7 years failed to identify neurotoxicity resulting from methyl chloroform exposure (Maroni *et al.*, 1977). The examination included evaluation for neurologic symptoms, changes in nerve conduction, and psychomotor tests.

Liver disease was observed in a worker exposed to methyl chloroform in a clothing factory screen printing room (Cohen and Frank, 1994). The worker was exposed for a total of 4 years before occupational exposure was identified as the cause of the liver disease. The worker sprayed an adhesive (containing 65% methyl chloroform, 25% propane and dimethyl ether and 10% inert ingredients) during which the worker reported often feeling dizzy or intoxicated. Three months following removal of the worker from exposure, liver function tests, although still abnormal, were significantly improved. Other case reports support these findings (Hodgson *et al.*, 1989; Halevy *et al.*, 1980).

Six male volunteers were exposed to 35 and 350 ppm methyl chloroform for 6-hours on two separate occasions (Nolan *et al.*, 1984). Absorption was determined to be 25% of the inhaled dose. Of the absorbed dose, 91% was excreted unchanged in the expired air. Although the odor was perceptible for the duration of the exposure, no subjective symptoms were reported by the volunteers.

## V. Effects of Animal Exposure

Gerbils (4/sex/dose plus 24 sex-matched control animals) were continuously exposed to 70, 210, or 1000 ppm methyl chloroform for 3 months (Rosengren *et al.*, 1985). A 4-month (solvent-free) recovery period following exposure was included to evaluate “lasting or permanent changes.” Body weights were not changed significantly as a result of exposure. Brain weights in the animals in the 1000 ppm dose group were significantly decreased. Fibrillary astrocytes are formed in the brain in response to injury. Brain injury in methyl chloroform exposed gerbils was evaluated by detection of glial fibrillary acidic (GFA) protein, the main protein subunit of astroglial filaments. Increased levels of GFA protein were detected in the sensorimotor cerebral cortex of animals exposed to 210 or 1000 ppm methyl chloroform.

A later study in gerbils examined the effects of a 3-month continuous exposure to 70 ppm methyl chloroform followed by a 4-month recovery period (Karlsson *et al.*, 1987). DNA content was significantly decreased in three areas of the brain: posterior cerebellar hemisphere, anterior cerebellar vermis, and hippocampus. The authors contended that depressions in DNA content reflect decreased cell density.

No evidence of peripheral neuropathy or other neurotoxicity was detected in rats exposed to 200, 620, or 2000 ppm methyl chloroform 6 hours per day, 5 days per week for 13 weeks (Mattson *et al.*, 1993). The study included a functional observational test battery and measured visual, somatosensory, auditory and caudal nerve-evoked potentials. Histopathology of the brain, spinal cord, peripheral nerves and limb muscles was also examined at the end of the 13-week exposure.

Forty percent of all mice continuously exposed to 1000 ppm methyl chloroform for 14 weeks exhibited evidence of hepatocellular necrosis (McNutt *et al.*, 1975). A statistically significant increase in liver weight per body mass was observed throughout the study. Electron microscopy revealed accumulation of triglyceride droplets in the centrilobular hepatocytes following one week of exposure to 1000 ppm methyl chloroform. After 4 weeks of exposure, cytoplasmic alterations in centrilobular hepatocytes included a loss of polyribosomes and increased smooth endoplasmic reticulum. Similar changes observed occasionally in hepatocytes from mice exposed to 250 ppm were not as dramatic.

Mild hepatocellular changes were observed in rats exposed to 1500 ppm methyl chloroform 6 hours per day, 5 days per week for 6, 12, and 18 months (Quast *et al.*, 1988). At 24 months, these slight effects were no longer discernible due to confounding geriatric changes. No hepatocellular changes or other adverse effects were observed in rats exposed to 150 or 500 ppm methyl chloroform for up to 24 months.

## VI. Derivation of Chronic Reference Exposure Level (REL)

<i>Study</i>	Rosengren <i>et al.</i> (1985)
<i>Study population</i>	Mongolian gerbils (4/sex/dose)
<i>Exposure method</i>	Whole-body inhalation exposure
<i>Critical effects</i>	Astrogliosis in the sensorimotor cerebral cortex
<i>LOAEL</i>	210 ppm
<i>NOAEL</i>	70 ppm
<i>Exposure continuity</i>	Continuous
<i>Average experimental exposure</i>	70 ppm for NOAEL group
<i>Human equivalent concentration</i>	70 ppm for NOAEL group (gas with systemic effects, based on RGDR = 1.0 using default assumption that $\lambda(a) = \lambda(h)$ )
<i>Exposure duration</i>	3 months
<i>LOAEL uncertainty factor</i>	1
<i>Subchronic uncertainty factor</i>	3
<i>Interspecies uncertainty factor</i>	10
<i>Intraspecies uncertainty factor</i>	10
<i>Cumulative uncertainty factor</i>	300
<i>Inhalation reference exposure level</i>	0.2 ppm (200 ppb; 1 mg/m <sup>3</sup> ; 1,000 µg/m <sup>3</sup> )

Case reports indicate that the nervous system and the liver are targets of the toxicity of methyl chloroform (House *et al.*, 1994; Liss, 1988; Howse *et al.*, 1989; Cohen and Frank, 1994). The largest of the epidemiological studies (Kramer *et al.*, 1978; Maroni *et al.*, 1977), however, did not identify adverse effects as a result of chronic methyl chloroform exposure. The Kramer *et al.* (1978) study limited its evaluation to changes in blood pressure, heart rate, or electrocardiogram and exposure levels were only characterized as less than 250 ppm. Maroni *et al.* (1977) conducted their study among 22 women exposed occupationally to methyl chloroform levels as low as 110 ppm. Although the subjects were evaluated specifically for signs of neurotoxicity, the small sample size limits conclusions which can be drawn from their failure to identify adverse effects in this population.

Data from animal studies generally support the findings of the case reports from human exposures. Both neurotoxicity and hepatotoxicity have been identified among animals exposed by inhalation to methyl chloroform. The adverse effect observed at the lowest level in these studies was the development of astrogliosis in the brains of gerbils exposed for 3 months to 210 ppm methyl chloroform (Rosengren *et al.*, 1985). A no-observed-adverse-effect-level (NOAEL) in this study was 70 ppm methyl chloroform. A subsequent study identified a more subtle change in the brains of gerbils exposed similarly to 70 ppm methyl chloroform, with slightly decreased DNA content found in several discrete brain regions of exposed animals. However, the relationship between tissue DNA content and cell density as an indication of adverse effect in the brain was considered too tenuous for the development of a guidance value for chronic exposure to methyl chloroform.

The major strengths of the REL are the observation of the NOAEL and the continuous subchronic exposure regimen. The major uncertainties are the lack of human exposure data, the lack of dose-response information, and the the lack of comprehensive multi-organ effects data.

## VII. References

ACGIH. 1992. American Conference of Governmental Industrial Hygienists, Inc. Documentation of the threshold limit values and biological exposure indices. Sixth edition. Cincinnati, OH.

ATSDR. 1990. Agency for Toxic Substances and Disease Registry. US Public Health Service. Toxicological profile for 1,1,1-trichloroethane. Prepared by Syracuse Research Corporation under contract to Clement Associates, Inc. under contract no. 205-88-0608.

Cohen C, and Frank AL. 1994. Liver disease following occupational exposure to 1,1,1-trichloroethane: a case report. *Am J Ind Med*, 26:237-41.

Halevy J, Pitlik S, Rosenfeld J, and Eitan B-D. 1980. 1,1,1-Trichloroethane intoxication: a case report with transient liver and renal damage. Review of the literature. *Clin Toxicol*, 16:467-72.

Hodgson MJ, Heyl AE, and Van Thiel DH. 1989. Liver disease associated with exposure to 1,1,1-trichloroethane. *Arch Intern Med*, 149:1793-8.

House RA, Liss GM, and Wills MC. 1994. Peripheral sensory neuropathy associated with 1,1,1-trichloroethane. *Arch Environ Health*, 49:196-9.

Howse DC, Shanks GL, and Nag S. 1989. Peripheral neuropathy following prolonged exposure to methyl chloroform. *Neurology*, 39:242.

HSDB. 1995. Hazardous Substances Data Bank. National Library of Medicine, Bethesda, Maryland (CD-ROM Version). Micromedex, Inc., Denver, Colorado (Edition expires 7/31/96).

Karlsson J-E, Rosengren LE, Kjellstrand P, and Haglid KG. 1987. Effects of low-dose inhalation of three chlorinated aliphatic organic solvents on deoxyribonucleic acid in gerbil brain. *Scand J Work Environ Health*, 13:453-8.

Kramer CG, Ott MG, Fulkerson JE, Hicks N, and Imbus HR. 1978. Health of workers exposed to 1,1,1-trichloroethane: a matched pair study. *Arch Environ Health*, 33:331-42.

Liss G. 1988. Peripheral neuropathy in two workers exposed to 1,1,1-trichloroethane. *JAMA*, 260:2217.

Maroni M, Bulgheroni C, Cassitto G, Merluzzi F, Gilioli R, and Foa V. 1977. A clinical, neurophysiological and behavioral study of female workers exposed to 1,1,1-trichloroethane. *Scand J Work Environ Health*, 3:16-22.

Mattson JL, Albee RR, Lomax LG, Beekman MJ, and Spenser PJ. 1993. Neurotoxicologic examination of rats exposed to 1,1,1-trichloroethane vapor for 13 weeks. *Neurotoxicol Teratol*, 15:313-26.

McLeod AA, Marjot R, and Monaghan MJ. 1987. Chronic cardiac toxicity after inhalation of 1,1,1-trichloroethane. *Br Med J*, 294:727-9.

McNutt NS, Amster RL, McConnell EE, and Morris F. 1975. Hepatic lesions in mice after continuous inhalation exposure to 1,1,1-trichloroethane. *Lab Invest*, 32:642-54.

Nolan RJ, Freshour NL, Rick DL, McCarty LP, and Saunders JH. 1984. Kinetics and metabolism of inhaled methyl chloroform (1,1,1-trichloroethane) in human volunteers. *Fundam Appl Toxicol*, 4:654-62.

Quast JF, Calhoun LL, and Frauson LE. 1988. 1,1,1-Trichloroethane formulation: a chronic inhalation toxicity and oncogenicity study in Fischer 344 rats and B6C3F1 mice. *Fundam Appl Toxicol*, 11:611-25.

Rosengren LE, Kjellstrand AA, and Haglid KG. 1985. Astrogliosis in the cerebral cortex of gerbils after long-term exposure to 1,1,1-trichloroethane. *Scand J Work Environ Health*, 11:447-55.

CHRONIC TOXICITY SUMMARY

# METHYL ETHYL KETONE

(2-Butanone; 3-butanone; methyl acetone; ethyl methyl ketone)

CAS Registry Number: 78-93-3

## I. Chronic Reference Exposure Level

<i>Inhalation reference exposure level</i>	<b>1,000 µg/m<sup>3</sup></b> (U.S. EPA RfC) This document summarizes the evaluation of non-cancer health effects by U.S. EPA for the RfC
<i>Critical effect(s)</i>	Decreased fetal birth weight in mice
<i>Hazard index target(s)</i>	Reproductive system

## II. Physical and Chemical Properties (HSDB, 1994)

<i>Description</i>	Colorless gas
<i>Molecular formula</i>	C <sub>4</sub> H <sub>8</sub> O
<i>Molecular weight</i>	72.10
<i>Specific gravity</i>	0.805 @ 20° C
<i>Boiling point</i>	79.6°C
<i>Melting point</i>	-86.3°C
<i>Vapor pressure</i>	77.5 torr @ 20° C
<i>Solubility</i>	Soluble in alcohol, ether, acetone, benzene and water
<i>Conversion factor</i>	1 ppm = 2.94 mg/m <sup>3</sup> @ 25° C

## III. Major Uses and Sources

Methyl ethyl ketone (MEK) is a solvent often found in mixtures with acetone, ethyl acetate, n-hexane, toluene or alcohols. MEK has applications in the surface coating industry and in the dewaxing of lubricating oils. MEK is utilized in the manufacture of colorless synthetic resins, artificial leather, rubbers, lacquers, varnishes, and glues (HSDB, 1994).

## IV. Effects of Human Exposures

Few reports of chronic human exposure to MEK were located in the literature. Peripheral neuropathy is described in case reports of workers occupationally exposed to mixtures of organic solvents including MEK (Dyro *et al.*, 1978; Billmaier *et al.*, 1974). Available animal data suggest a possible synergistic action between MEK and some organic solvents.

## V. Effects of Animal Exposures

Chronic respiratory disease was observed in rats of all groups exposed to 1254, 2518, or 5041 ppm MEK 6 hours per day, 5 days per week for 90 days (Cavender *et al.*, 1983; Toxigenics, 1981). General histological examination was performed on 10 animals from each exposure group and neuropathologic examination was performed on the remaining 5 animals from each exposure group. High prevalences of nasal inflammation were observed in all exposure groups and in controls; the authors therefore suggest that the pulmonary lesions were a result of mycoplasma infection although no infectious agent was cultured. Increased relative kidney and liver weights were observed in rats exposed to 5041 ppm MEK. Female rats exposed at this level also exhibited an increase in serum alkaline phosphatase levels. No pulmonary or neurologic functional tests were conducted.

No adverse effects were observed in pregnant rats exposed to 1126 or 2618 ppm MEK 7 hours per day on days 6-15 of gestation (Schwetz *et al.*, 1974). A statistically significant increase in the number of litters with fetuses with skeletal anomalies was observed in the offspring of the exposed rats as compared to controls. No single soft tissue anomaly was observed at a statistically significant increased incidence.

A more recent study exposed groups of about 30 pregnant mice to 0, 400, 1000, or 3000 ppm MEK 7 hours per day on days 6-15 of gestation (Schwetz *et al.*, 1991). A slight, statistically-significant increase in maternal liver weight was observed in the 3000 ppm exposure group. No overt signs of maternal toxicity were observed. Decreased fetal body weight was observed following maternal exposure to 3000 ppm MEK. The reduction in fetal body weight was statistically significant in male offspring only. Cleft palate, fused or missing sternabrae and syndactyly were observed at low incidences in all groups other than controls. There was also a significant trend for increased incidence of misaligned sternabrae.

Possible synergistic effects of combined exposures to MEK and n-hexane were examined in groups of 8 rats exposed to 100 ppm n-hexane, 200 ppm MEK, 100 ppm n-hexane plus 200 ppm MEK, or fresh air in a chamber for 12 hours per day for 24 weeks (Takeuchi *et al.*, 1983). Motor nerve conduction velocity (MCV), distal motor latency (DL) and mixed nerve conduction velocities (MNCVs) were measured at 0, 4, 8, 12, 16, 20, and 24 weeks of exposure. After 4 weeks of exposure rats in the 200 ppm MEK groups exhibited significant increases in MCV and MNCV and a significant decrease in DL. No significant differences were observed in subsequent weeks in this exposure group. In the 100 ppm n-hexane group, a significant decrease was observed in DL after 4 weeks and a slight non-significant decrease was observed in MNCV after 24 weeks. Rats exposed to 100 ppm n-hexane plus 200 ppm MEK exhibited significant decreases in MCV, MNCV after 20 and 24 weeks of exposure suggesting that mixed exposure to n-hexane and MEK may be more toxic than n-hexane alone.

## VI. Derivation of U.S. EPA Reference Concentration

<i>Study</i>	Schwetz <i>et al.</i> , 1991
<i>Study population</i>	Mice
<i>Exposure method</i>	Inhalation on days 6-15 of gestation
<i>Critical effects</i>	Decreased mean fetal body weight
<i>LOAEL</i>	3,020 ppm
<i>NOAEL</i>	1,010 ppm
<i>Exposure continuity</i>	7 hours/day
<i>Average experimental exposure</i>	1,010 ppm for NOAEL group (time-weighted exposure not used by U.S. EPA for reproductive effects)
<i>Human equivalent concentration</i>	1,010 ppm for NOAEL group (gas with systemic effects, based on RGDR = 1.0 using default assumption that $\lambda(a) = \lambda(h)$ )
<i>Exposure duration</i>	7 hours per day on days 6-15 of gestation
<i>LOAEL uncertainty factor</i>	1
<i>Subchronic uncertainty factor</i>	1
<i>Interspecies uncertainty factor (UF)</i>	10
<i>Intraspecies uncertainty factor (UF)</i>	10
<i>Modifying factor</i>	10
<i>Cumulative uncertainty factor</i>	1,000
<i>Inhalation reference exposure level</i>	0.3 ppm (300 ppb; 1 mg/m <sup>3</sup> ; 1,000 µg/m <sup>3</sup> )

While there are case reports indicating neurotoxicity following long-term low-level exposures to organic solvents, adequate quantitative data describing the neurotoxic action of MEK were not located. Therefore, developmental toxicity was identified from the available data as the most sensitive endpoint for long-term exposures.

The major strengths of the REL are the observation of a NOAEL, and the use of data with controlled and quantified exposure. The major uncertainties are the lack of human data and the lack of long-term exposure data.

## V. References

Billmaier D, Yee HT, Allen N, Craft B, Williams N, Epstein S, Fontaine R. 1974. Peripheral neuropathy in a coated fabrics plant. *J. Occ. Med.* 16(10):665-671.

Cavender FL, Casey HW, Salem H, Swenberg JA, and Gralla EJ. 1983. A 90-day vapor inhalation toxicity study of methyl ethyl ketone. *Fund. Appl. Toxicol.* 3(4):264-270.

Dyro M. 1978. Methyl ethyl ketone polyneuropathy in shoe factor workers. *Clinical Tox.* 13(3):371-376.

HSDB. 1993. Hazardous Substance Data Bank National Library of Medicine, Bethesda, Maryland (CD-ROM Version). Micromedex, Inc., Denver, Colorado (Edition expires 11/31/93).

Schwetz BA, Leong BKJ, and Gehring PJ. 1974. Embryo- and fetotoxicity of inhaled carbon tetrachloride, 1,1-dichloroethane and methyl ethyl ketone in rats. Toxicol. Appl. Pharm. 28:452-464.

Schwetz BA, Mast TJ, Weigel RJ, Dill JA, Morrissey RE. 1991. Developmental toxicity of inhaled methyl ethyl ketone in Swiss mice. Fund. Appl. Toxicol. 16:742-748.

Takeuchi Y, Ono Y, Hisanaga N, Iwata M, Aoyama M, Kitoh J, and Sugiura Y. 1983. An experimental study of the combined effects of n-hexane and methyl ethyl ketone. Br. J. Ind. Med. 40:199-203.

Toxigenics 1981. 90-Day vapor inhalation toxicity study of methyl ethyl ketone in albino rats. Submitted to Chemical Industry Institute of Toxicology, Research Triangle Park, NC. Doc ID 878212064, Microfiche No. 205953. [cited in U.S. EPA, 1994].

U.S. EPA. 1994. U. S. Environmental Protection Agency. Integrated Risk Information System (IRIS). Office of Health and Environmental Assessment, Environmental Criteria Assessment Office, Cincinnati, OH.

CHRONIC TOXICITY SUMMARY

METHYL ISOCYANATE

(MIC,  $\text{CH}_3\text{-N}=\text{C}=\text{O}$ )

CAS Registry Number: 624-83-9

I. Chronic Toxicity Summary

<i>Inhalation reference exposure level</i>	1 $\mu\text{g}/\text{m}^3$
<i>Critical effects(s)</i>	Decreased weight gain and lung pathology at cessation of exposure in rats
<i>Hazard index target(s)</i>	Respiratory system; reproductive system

II. Chemical Property Summary (HSDB, 1995)

<i>Molecular formula</i>	$\text{C}_2\text{H}_3\text{NO}$
<i>Molecular weight</i>	57.06
<i>Description</i>	Colorless liquid
<i>Vapor pressure</i>	348 mm Hg @ 20°C, 600 mm Hg @ 30°C (Varma and Guest, 1993)
<i>Solubility</i>	10 percent in water @ 15°C
<i>Conversion factor</i>	2.3 $\mu\text{g}/\text{m}^3$ per ppb at 25°C

III. Major Uses and Sources (Dave, 1985; U.S. EPA, 1986; HSDB, 1995)

Methylisocyanate (MIC) is prepared industrially by reacting methylamine with phosgene, oxidizing monomethylformamide at high temperatures ( $\geq 550^\circ\text{C}$ ), or heating metal methylisocyanates. Because of its high reactivity, MIC is used as an intermediate in organic synthesis, most notably, in the production of carbamate based pesticides. Tobacco smoke from some brands of cigarettes also contain MIC (about 4  $\mu\text{g}$  per cigarette). Workers exposed to the MIC 8-hour threshold limit value (0.02 ppm) are exposed to approximately 460  $\mu\text{g}$  MIC in a workday.

IV. Effects of Human Exposure

Although occupational exposures to MIC have been documented (Varma, 1986), few known exposures to the general public have occurred. A major exposure occurred in Bhopal, India in 1984. Because of the sudden and short release (30 -45 minutes), no measurements occurred and the air concentration was estimated as 13 ppm. (Dave, 1985) to 100 ppm (Varma, 1986).

The chemical identity of the ultimate toxicant has yet to be unequivocally determined and may consist of more than one chemical species. Although the chemistry of MIC suggests hydrolysis to methylamine and dimethylurea is rapid, such hydrolysis in moist air is probably slow, and the reaction with photochemically produced hydroxyl radical is also slow (chemical  $T_{1/2}$  about 3 months) (U.S. EPA, 1986). Brown *et al.* (1987) have shown that the alkylisocyanates (e.g. MIC) are relatively resistant (compared to the arylisocyanates) to hydrolysis in water. Hence, despite the high water reactivity of MIC, this compound could possibly persist in the environment for many days after an initial release.

Within 5-days of the initial exposure to MIC at Bhopal, more than 2,000 deaths occurred (Dave, 1985), while 4,000 more deaths have been documented during the intervening decade (Lepkowski, 1994). The initial symptoms experienced by the population living near the MIC plant, were irritation and difficulty in breathing (Varma, 1986). Blindness occurred in more than 10,000 exposed persons but later resolved in most cases (Andersson *et al.*, 1990). The acute damage that led to death was mainly to the respiratory system, most likely pulmonary edema, bronchospasm, and electrolyte imbalance (Varma, 1986). However extrapulmonary damage, including tissue anoxia, gastrointestinal symptoms and muscular weakness were also observed (Dave, 1985). Within a year of the exposure, survivors continued to exhibit damage to the lung and eyes. Fibrosis of the lungs was seen in 30 percent of this group. (Dave, 1985).

Reproductive toxicity was observed among women exposed to MIC in Bhopal. Varma (1987) reported 43 percent unsuccessful pregnancies among 865 women who were pregnant at the time of the MIC release. Among the live births, 14 percent of the infants died within 30 days, whereas a death-rate of only 3 percent for the same interval was recorded 2-years prior to the release. Bhandari *et al.* (1990) reported increased spontaneous abortions and neonatal deaths among exposed women who were pregnant at the time of exposure compared to a control group in another city. In the latter study, still births and congenital malformations were similar in the exposed and non-exposed groups.

Non-reproductive, non-pulmonary responses were evident in a group of exposed Bhopal residents, 3-years following exposure to the MIC vapors. Loss of vision and loss of visual acuity were more prominent among exposed residents than among unexposed people, and the losses appeared to be dose-dependent (Andersson *et al.*, 1990). In this study, the surrogate for dose was extent of early deaths in a housing cluster. Similarly, cataracts were reported more often among the exposed than among the unexposed group.

The lesions associated with lung damage may be expressed as pulmonary edema for immediate effects (Varma, 1986), and lesions associated with the bronchoalveolar area for long-term effects (Dave, 1985, Varma, 1986). Vijayan *et al.* (1995) studied cellular components of bronchoalveolar lavage (BAL) and pulmonary function in Bhopal patients 1.3-, 2.7-, and 5.1-years after exposure to MIC. All had lived within 3-miles of the factory and all experienced acute respiratory and ophthalmic symptoms on the day of exposure. All were experiencing continued respiratory symptoms. Among the exposed people, decrements in forced vital capacity and forced expiratory volume (at 1-minute) were observed. In general, the decrements ranged

from 12 - 21 percent of predicted values whereas the control group exhibited decrements of 2 - 4 percent of the expected values. Analysis of the BAL revealed increases in total cells (all exposed groups), increased absolute numbers of macrophages (all exposure groups), decreased percentage of lymphocytes (2.7- and 5.1- year groups), and increased numbers and percentage of neutrophils (5.1- year group). These cell types are involved, through the secretion of various factors, in inflammatory and immunologic processes in the lung (Reiser and Last, 1986). The Vijayan *et al.* (1995) study thus suggests a long term damage to lung parenchyma among people who survived the initial acute effects of MIC exposure.

In summary, humans exposed acutely by inhalation to MIC may experience long-term (as well as immediate) damage to pulmonary and extrapulmonary systems. In terms of cause of death, the lung is probably the critical target organ for long-term effects from acute exposure, although adverse effects on other organs (e.g. eye, reproductive, and gastrointestinal) also exist. The late responses to the acute exposure suggest an immunological component which could involve several systems, including lung, eye, liver, and kidney. The chemical identity of the ultimate toxicant is unknown and may be represented by more than one compound.

## **V. Effects of Animal Exposure**

Experimental animal studies have been designed to address the experiences of the victims of the Bhopal disaster, in which the exposure has been described as acute because of the short duration (30-45 min). No studies were found that described exposure durations greater than 10-days. However, a chronic component to MIC exposure may exist as a result of slower rates of hydrolysis in air (compared to water), the presence of carbamylated hemoglobin in MIC-exposed people, and the change from edematous to inflammatory and/or fibrotic lesions with time. Further, a glutathione-dependent reversible MIC transport system has been suggested in experimental animals (see below).

MIC is absorbed through the respiratory tract and distributed to non-respiratory organs in experimental animals. In an acute (30 min) inhalation exposure to a dose of  $^{14}\text{C}$ -MIC, (isocyanate moiety), equivalent to one-LC<sub>50</sub> (23 mg/L), rats accumulated protein-bound radioactivity (including carbamylated proteins) in brain, liver, and kidney, and lung, but not in blood (Bhattacharya *et al.*, 1988). Ferguson *et al.* (1988) exposed guinea pigs by inhalation to 0.47 ppm  $^{14}\text{C}$ -MIC (methyl group) for 6-hours. At the end of exposure, the label was found in arterial and venous blood, bile, and urine. At 2.7 days post-exposure, the label decreased to 2-7 percent. MIC was retained in the nasal-laryngeal area of the guinea pigs.

MIC, like reactive isocyanates in general, can react with biological molecules containing amino, alcohol, or sulfhydryl groups, as well as with water. While hydrolysis in an aqueous environment, such as the lung, is theoretically possible, measurements show that alkyl isocyanates are relatively resistant (compared to arylisocyanates) to such hydrolysis (Brown *et al.* 1987). The absence of a role for MIC hydrolytic products, methylamine (MA) or dimethylurea (DMU), is also suggested by the work of Jeevaratnam and Sriramachari (1994), wherein inhalation (30 min) or subcutaneous exposure to rats to either hydrolytic product at levels

equivalent to the LC<sub>50</sub> or LD<sub>50</sub> did not result in death. Similarly, the acute effects of respiratory necrosis and congestion was not duplicated by MA or DMU. However, exposure to these hydrolytic products did lead to interstitial pneumonitis, an observation that suggests MA and/or DMU could lead to subsequent inflammatory responses if sufficient amounts are present.

A role for MMA in reproductive / developmental toxicity was investigated by Guest and Varma (1991). In a mouse study, pregnant dams were exposed to varying doses (ip) of MMA as well as the di- and trimethyl compounds. Reproductive toxicity was not observed for the MMA. However, in cultured embryo experiments, decrements in crown-rump length, yolk-sac diameter, head length, and embryo survival was observed. The concentrations were high (>0.75 mM) and the interpretation of the biological activity of MMA in terms of inhalation exposure is difficult.

MIC is a carbamylating intermediate; this is the basis for its use in the manufacture of carbamate based pesticides. In the same way, MIC should react with the appropriate functional groups of proteins, peptides, and nucleic acids. *In vitro* studies with cholinesterases, however show such a reaction is not efficient (Brown *et al.*, 1987), an observation which may be explained by presence of protonated amino groups at physiological pH (Baillie and Slatter, 1991). Nonspecific interactions, however, were described by Brown *et al.* (1987) who reported that the reversal of cholinesterase activity, previously inhibited to 28 percent by an isocyanate, did not occur when the original MIC inhibition was below 28 percent. These considerations indicate MIC related symptoms are multifactorial.

A transport system for MIC via reduced glutathione (GSH) has been suggested by the discovery of the MIC-adduct, S-(N-methylcarbamoyl)glutathione (SMG) in the bile and the MIC-adduct of N-acetylcysteine (mercapturic acid, AMCC) in the urine of rats exposed to MIC by non-inhalation routes (Pearson *et al.*, 1990; Slatter *et al.*, 1991). The reaction of MIC with GSH and with cysteine is reversible, and can provide a source of free MIC in the tissues (Baillie and Slatter, 1991). Similar studies in experimental animals exposed to MIC by inhalation have not been reported. However, humans exposed by inhalation to N,N-dimethylformamide (H-C(=O)-N(CH<sub>3</sub>)<sub>2</sub>) excrete AMCC in urine (Mraz and Nohova, 1992). Hence a reversible MIC-transport system in animals, including humans, is possible, and the presence of high levels of GSH in human lavage fluid (Cantin *et al.*, 1987) would permit the initiation of this mechanism.

The toxicity of the adduct, SMG, was tested in mouse embryo culture (Guest *et al.*, 1992). Mouse embryos, at day 8 gestation, were removed from mice and cultured in the presence (and absence) of SMG. Dose-dependent (0.25 - 2 mM) decrements were observed for yolk sac diameter, crown-rump length, somite number and protein content. Delayed DNA synthesis in the embryos and in yolk-sacs occurred in the presence of 0.25 mM SMG. Similar to the results obtained with MMA, the SMG concentrations were high and the exposures were not inhalation. However, the data show that a MIC metabolite, SMG, has toxic properties. In the presence of GSH (1 or 3 mM), the extent of the SMG - dependent toxicities were decreased. Such data demonstrate the reversibility of the binding between MIC and GSH.

Three inhalation studies were identified in which experimental animals were exposed to more than one dose of MIC. Among these studies, two used exposure durations for more than one day

(Dodd and Fowler, 1986; Mitsumori *et al.*, 1987); rats and mice were exposed by inhalation to 0, 1.1, and 2.8 (female) or 3.0 (male) ppm MIC for 6 hr/day for a total of 4 days, and then followed during a 91 day post-exposure interval (Mitsumori *et al.*, 1987). Among the rats, post-exposure deaths occurred by 49 days (male) and 14 days (female) at the high dose. Among the mice, only 1 male mouse died at 16 days post-exposure. Reduced weight gain was observed among the female and male rats in the high dose group, prior to death, although the absolute weights were not different from the unexposed rats one day before the end of exposure. Among the mice, a weight gain loss was observed at 3- and 6-days post exposure (male) and 1 day post exposure (female) at the high dose, but normal weight gain returned by 1-week following cessation of exposure. At 7 days post-exposure, microscopic changes were observed in the respiratory system among the high dose rats of both sexes. Between 8- and 27 days post-exposure increased lesions in the respiratory tract and also in liver, thymus, spleen and heart, and brain were observed at the high dose. Similar lesions were not observed in rats exposed to 1.1 ppm MIC and followed to the 8-27 day post-exposure. Among survivors, the incidence of lesions decreased to control values by 91 days. Among the mice, treatment related changes in the respiratory tract were observed at high dose at 7 days post-exposure. Between 28 and 91 days, the lesions associated with the upper respiratory tract disappeared, whereas those associated the major bronchi remained, although somewhat attenuated. These data suggest the rat is more sensitive than mouse to the effects of MIC. A LOAEL of 2.9 ppm is indicated, based on post-exposure decreased weight gain and respiratory tract changes in rats.

Dodd and Fowler (1986) exposed rats to 0, 0.15, 0.6, and 3.1 ppm MIC for two 4-day sessions at 6- hours/day and examined the animals within 1-day following exposure. The 2-cycle exposure included a 2-day recess from exposure. No deaths occurred at any MIC concentration during the exposure. Weight gain loss occurred for rats in the 3.1 ppm groups, whereas weight among the rats in the 0.15 and 0.6 ppm MIC groups was indistinguishable from the air-exposed control animals. On exposure days 3 and 8, mean food consumption values in the high dose group were below those for the non-exposed group. At the time of sacrifice, male rats exposed to 3.1 ppm MIC, exhibited a 38 percent increase ( $p<0.001$ ) in hemoglobin concentration and a 26 percent decrease ( $p<0.001$ ) in oxygen saturation, compared to the unexposed rats. Such changes were not observed for the female rats exposed to 3.1 ppm or for rats of either sex exposed to 0.15 or 0.6 ppm. Absolute lung weights increased ( $p<0.001$ ) among both sexes in the 3.1 ppm exposure group, compared to the control rats. Decreases in liver, kidney and testes absolute weights were observed in this exposure group, but these data were interpreted by the authors as a reflection of the body weight losses. No weight changes were observed in rats exposed to 0.15 or 0.60 ppm MIC. Gross and microscopic lesions were observed in rats (female and male) exposed to 3.1 ppm, but not in rats exposed to 0, 0.15, or 0.6 ppm MIC. The microscopic lesions occurred in the respiratory tract and consisted of inflammation, epithelial necrosis, squamous metaplasia, and epithelial hyperplasia. These lesions extended into the bronchioles. These data suggest a NOAEL of 0.6 ppm MIC, based on weight gain loss, absolute lung weight and lung histopathology in rats, immediately following cessation of exposure.

Post-exposure changes in lung pathology also occurred in the 3.1 ppm surviving rats in the Dodd and Fowler (1986) study. The early lesions associated with inflammation, epithelial necrosis, squamous metaplasia, and epithelial hyperplasia extending to the bronchioles either decreased in

severity or receded toward the upper respiratory tract by 85-days post-exposure. In males, the intraluminal and submucosal fibroplasia changed in appearance during this interval, due in part to the maturation of fibrous tissue. Mucous plugs were also seen in the terminal bronchioles and alveoli in some rats. The importance of this observation is the progressive character of MIC induced lung disease. Such progression may be difficult to follow at lower doses, if the times involved are beyond experimental capability.

Sethi *et al.* (1989) exposed rats by inhalation to 0, 0.21, 0.26, and 0.35 ppm MIC for 6-days at 0.5 hr / day. Statistical evaluation was not presented. No post-exposure deaths were reported, although lethality was recorded for rats exposed to 3.5 and 35 ppm for only 10 minutes. Following the 0.5 hr x 6-day exposure, the weight gain declined in proportion to the exposure dose. At the lowest dose (0.21 ppm) the weight gain was 111 gm after 91 days post-exposure, compared to a weight gain of 218 gm during the same interval among the non-exposed rats. The absolute weights of the rats at the end of the exposure were not given. According to the narrative, inflammatory lesions of bronchopulmonary tissue were present, the extent of which increased with dose. A dose-response increase in markers of lung infection were present and suggest the MIC exposed rats were more prone to infectious agents than were the unexposed animals. Non-specific lesions in liver and kidneys were also observed and appeared to be dose dependent, but the authors suggest these effects could be a result of the lung infections.

Fetotoxicity was observed in two experimental animal studies (Schwetz *et al.*, 1987; Varma, 1987). Among female mice exposed to 0, 1, or 3 ppm MIC during gestation days 14 - 17 for 6 hr/day, an increased incidence of fetal deaths was observed at 1 ppm (Schwetz *et al.*, 1987). At 3 ppm, the average number of pups/ litter decreased relative to the air-exposed controls. The dams were unaffected in terms of survival, body weight, or length of gestation. Non-gestational exposure (6 hr/day, 4-days) did not affect the number of pregnancies or the live litter sizes, suggesting the fetotoxic effect may be specific to the female reproductive tract rather than a general attribute of systemic toxicity. Similarly, female mice exposed for 3 hours on gestation day-8 to 0, 2, 6, or 9 ppm MIC, gave birth to pups with decreased body weights at the lowest dose, although a good dose-response was not observed (Varma, 1987). At 9 or 15 ppm MIC, the surviving dams lost 75 - 80 percent of their fetuses. Maternal mortality and decreased skeletal lengths were also observed at 9 and 15 ppm. A distinction between maternally induced fetotoxicity and a direct effect on fetal health could not be made. Because the inhalation exposure to the dams occurred for only 3-hrs on one day, a chronic LOAEL is not suggested. Exposure of male rats to one dose of 3.2 mg/L for 8-min resulted in a 21 percent fertility rate among the cohabited female rats within the first 14 days of post-exposure, but the rates increased after 15 days post-exposure (Agarwal and Bose, 1992). There was no evidence of fetotoxicity among the dams impregnated by the MIC-exposed male rats. Exposure of male and female mice to 0, 1, or 3 ppm MIC did not result in altered body weights, fertility or litter size (Schwetz *et al.*, 1987). The results suggest that exposures to MIC at doses that are not toxic to adult male or female (pregestational) mice or rats do not result in adverse reproductive outcomes.

## **VI. Derivation of Chronic Reference Exposure Level (REL)**

<i>Study</i>	Dodd and Fowler (1986)
<i>Study populations</i>	F344 rats
<i>Exposure method</i>	Inhalation (0, 0.15, 0.6, or 3.1 ppm)
<i>Critical effects</i>	Decreased weight gain and lung pathology at immediate cessation of exposure
<i>LOAEL</i>	3.1 ppm
<i>NOAEL</i>	0.6 ppm
<i>Exposure continuity</i>	6 hours/day, 4 days/wk (2-cycles, with one 2-day recess from exposure)
<i>Exposure duration</i>	11 days
<i>Average experimental exposure</i>	0.11 ppm for the NOAEL group
<i>Human equivalent factor</i>	0.135 ppm for the NOAEL group (gas with pulmonary respiratory effects, RGDR = 1.23, based on BW = 152 g, MV = 0.12 L/min, SA = 225 cm <sup>2</sup> )
<i>LOAEL uncertainty factor</i>	1
<i>Subchronic factor</i>	10
<i>Interspecies uncertainty factor</i>	3
<i>Intraspecies uncertainty factor</i>	10
<i>Cumulative uncertainty factor</i>	300
<i>Inhalation reference exposure level</i>	0.5 ppb (1 µg/m <sup>3</sup> )

The Dodd and Fowler (1986) study includes the longest exposure duration of the available investigations and also uses some of the lower exposure levels (down to 0.15 ppm). The microscopic findings of the respiratory tract were statistically analyzed, although an observation of the tabulated data at the four doses (0, 0.15, 0.6, and 3.1 ppm) clearly shows a NOAEL of 0.6 ppm. Other endpoints with the same NOAEL were increased hemoglobin and increased absolute lung weights. The symptomatic ramifications of the increased hemoglobin is unknown, although similar increases were reported for humans exposed to MIC in Bhopal (Srivastava *et al.*, 1988). The lung weight gain may be a reflection of the pathological changes seen in the microscopic studies.

Decreased body weight gain was also seen in the experimental 4-day rat inhalation study of Mitsumori *et al.* (1987) (NOAEL = 1.0 ppm), except that the decrease in the latter study did not occur until 1- and 3-days (female and male respectively) post-exposure. The apparent discrepancy could be explained, in part, on the basis of the length of exposure, which was twice as long in the Dodd and Fowler (1986) study. However, the weight gain loss in the Dodd and Fowler (1986) study was initiated within one day of the start of exposure.

The MIC chronic REL of 0.4 ppb is based on endpoints observed within 1-day of cessation of exposure. Post-exposure evaluation showed that at a higher exposure level (3.1 ppm), progressive changes, including death, occurred. Post-exposure observations, however, were not reported at the 0.15, and 0.6 ppm MIC levels. The attribute of delayed MIC inhalation toxicity has also been observed in other experimental animals studies (Fowler and Dodd, 1986, Mitsumori *et al.*, 1987). In the case of the human MIC exposure in Bhopal India, death did not

occur during the immediate 30 - 45 minute exposure, but exhibited a lag phase. A few deaths occurred during the first few hours, the maximum occurred at 2 - 3 days and by the end of a week, about 2500 deaths were documented (Dave, 1985; Varma, 1986; Varma and Guest, 1993), although Varma (1986) suggests the immediate number may be closer to 5,000. One report suggests that during the intervening decade, as many as 6,000 deaths may be attributed to the initial exposure in Bhopal (Lepkowski, 1994). Such information suggests that the presence of an adverse effect at the NOAEL of 0.6 ppm (Dodd and Fowler, 1986) might be possible if the rats were observed during an extended post-exposure interval. Experimental evidence is needed to test this hypothesis.

Only one study was identified in which post-exposure observations were made on experimental animals exposed subchronically by inhalation to multi-doses of MIC. Mitsumori *et al.* (1987) exposed rats to 0, 1.1, and 2.9 ppm MIC for 6 hr/day for 4-days and observed the rats for 91 days. No deaths and no weight gain loss (compare Dodd and Fowler, 1986) were present until the post-exposure period at 2.9 ppm. Using a NOAEL of 1.1 ppm MIC, a chronic REL of 0.28 ppb was derived. The REL based on the Mitsumori *et al.* (1987) study is similar to the REL based on immediate effects (Dodd and Fowler, 1986), and may indicate the time of occurrence of exposure related effects may not be as important as the MIC air concentration.

The strengths of the inhalation REL include the availability of controlled exposure inhalation studies in multiple species at multiple exposure concentrations and with adequate histopathological analysis and the observation of a NOAEL. Major areas of uncertainty are the lack of adequate human exposure data and the lack of chronic inhalation exposure studies.

## VII. References

- Agarwal DK, and Bose M. 1992. Inhalation toxicity of methylisocyanate: assessment of germ cell mutagenicity and reproductive effects in rats. *Ind. J. Exp. Biol.* 30:504-508.
- Andersson N, Ajwani MK, Mahashabde S, Tiwari MK, Muir MK, Mehra V, Ashiru K, and Mackenzie DC. 1990. Delayed eye and other consequences from exposure to methyl isocyanate: 93% follow up of exposed and unexposed cohorts in Bhopal. *Br. J. Ind. Med.* 47:553-558.
- Baillie TA, and Slatter JG. 1991. Glutathione: a vehicle for the transport of chemically reactive metabolites *In Vivo*. *Acc. Chem. Res.* 24:264-270.
- Bhandari NR, Syal AK, Kambo I, Nair A, Beohar V, Saxena NC, Dabke AT, Agarwal SS, and Saxena BN. 1990. Pregnancy outcome in women exposed to toxic gas at Bhopal. *Ind. J. Med. Res.* [B]. February. pp 28-33.
- Bhattacharya BK, Sharma SK, and Jaiswal DK. 1988. *In Vivo* Binding of [1-<sup>14</sup>C]Methylisocyanate to various tissue proteins. *Biochem. Pharmacol.* 37:2489-2493.

Brown WE, Green AH, Cedel TE, and Cairns J. 1987. Biochemistry of protein-isocyanate interactions: a comparison of the effects of aryl vs. alkyl isocyanates. *Environ. Health Persp.* 72:5-11.

Cantin AM, North SL, Hubbard RC, and Crystal RG. 1987. Normal alveolar epithelial lining fluid contains high levels of glutathione. *J. Appl. Physiol.* 63:152-157.

Chandra H, Saraf AK, Jadhav RK, Rao GJ, Sharma VK, Sriramachari S, and Vairamani M. 1994. Isolation of an unknown compound, from both blood of bhopal aerosol disaster victims and residue of tank E-610 of union carbide India limited-chemical characterization of the structure. *Med. Sci. Law.* 34:106-110.

Dave JM. 1985. The bhopal methyl isocyanate (MIC) incident: an overview. In: *Proceedings of an International Symposium, Highly Toxic Chemicals: Detection and Protection Methods*. Schiefer, H.B. ed. Saskatoon, Saskatchewan, Canada. pp 1-38.

Dodd DE, and Fowler EH. 1986. methyl isocyanate subchronic vapor inhalation studies with Fischer 344 rats. *Fund. Appl. Toxicol.* 7:502-522.

Ferguson JS, Kennedy AL, Stock MF, Brown WE, and Alarie Y. 1988. Uptake and distribution of  $^{14}\text{C}$  during and following exposure to [ $^{14}\text{C}$ ] methyl isocyanate. *Toxicol. Appl. Pharmacol.* 94:104-117.

Guest I, Baille TA, and Varma DR. 1992. Toxicity of the methyl isocyanate metabolite s-(n-methylcarbamoyl)GSH on mouse embryos in culture. *Teratology.* 46:61-67.

Guest I, and Varma DR. 1991. Developmental toxicity of methylamines in mice. *F. Toxicol. Environ. Health.* 32:319-330.

HSDB. 1995. Hazardous Substances Data Base. Micromedex, Inc. Vol. 25. Expires 07/31/95.

Jeevaratnam K, and Sriramachari S. 1994. Comparative toxicity of methyl isocyanate and its hydrolytic derivatives in rats. I. Pulmonary Histopathology in the Acute Phase. *Arch. Toxicol.* 69:39-44.

Lepkowski W. 1994. Then years later - Bhopal. *Chemical and Engineering News.* 19 December 1994.

Mitsumori K, Boorman GA, Gupta BN, and Bucher JR. 1987. Four-day repeated inhalation and recovery study of methyl isocyanate in F344 rats and B6C3F1 mice. *Fund. Appl. Toxicol.* 9:480-495.

Mraz J, and Nohova H. 1992. Absorption, metabolism and elimination of N,N-dimethylformamide in humans. *Int. Arch. Occup. Environ. Health.* 64:85-92.

Pearson PG, Slatter JG, Rashed MS, Han D-H, Grillo MP, and Baillie TA. 1990. S-(N-Methylcarbamoyl)glutathione: a reactive s-linked metabolite of methyl isocyanate. *Biochem. Biophys. Res. Comm.* 166:245-250.

Reiser KM, and Last JA. 1986. Early cellular events in pulmonary fibrosis. *Exp. Lung Res.* 10:331-355.

Schwetz BA, Adkins BFr, Harris M, Moorman M, and Sloane R. 1987. Methyl isocyanate: reproductive and developmental toxicology studies in mice. *Environ. Health Perspect.* 72:1490152.

Sethi N, Dayal R, and Singh RK. 1989. Acute and subacute toxicity study of inhaled methyl isocyanate in charles foster rats. *Ecotoxicol. Environ. Saf.* 18:68-74.

Slatter JG, Rashed MS, Pearson PG, Han D-H, and Baillie TA. 1991. Biotransformation of methyl isocyanate in the rat. Evidence of glutathione conjugation as a major pathway of metabolism and implications for isocyanate-mediated toxicities. *Chem. Res. Toxicol.* 4:157-161.

Sriramachari S, Rao GJ, Sharma VK, Jadhav RK, Saraf AK, and Chandra H. 1991. GC-NPD and GC-MS analysis of preserved tissue of Bhopal gas disaster: evidence of methyl carbamylation in oost-mortem blood. *Med. Sci. Law.* 31:289-293.

Srivastava RC, Gupta BN, Athar M, Behari JR, Dwivedi RS, Hasan SK, Bharti RS, Singh A, Misra M, and Ray PK. 1988. Effect of exposure to toxic gas on the population of Bhopal: Part III - assessment of toxic manifestations in humans - hematological and biochemical studies. *Ind. J. Exp. Biol.* 26:165-172.

U.S. EPA. 1986. United States Environmental Protection Agency. Health and environmental effects profile for methyl isocyanate. EPA/600/22. Environmental Criteria and Assessment Office, Office of Research and Development. Cincinnati OH.

Varma DR. 1986. Anatomy of the methyl isocyanate leak on Bhopal. In: Hazard Assessment of Chemicals. J. Saxena, ed., Hemisphere Publishing Corp. New York. pp. 233-299.

Varma DR. 1987. Epidemiological and experimental studies on the effects of methyl isocyanate on the course of pregnancy. *Environ. Health Perspect.* 72:153-157.

Varma DR, and Guest I. 1993. The Bhopal accident and methyl isocyanate toxicity. *J. Toxicol. Environ. Health.* 40:513-529.

Vijayan VK, Pandey VP, Sankaran K, Mehotra Y, Darbari BS, and Misra NP. 1989. Bronchoalveolar lavage study in victims of toxic gas leak at Bhopal. *Ind. J. Med. Res.* 90:407-414.

Vijayan VK, Sankaran K, Sharma SK, and Misra NP. 1995. Chronic lung inflammation in victims of toxic gas leak at Bhopal. *Resp. Med.* 89:105-111.

CHRONIC TOXICITY SUMMARY

# METHYL METHACRYLATE

(2-(methoxycarbonyl)-1-propene; methyl 2-methacrylate; methyl 2-methyl-2-propenoate; MMA)

CAS Registry Number: 80-62-6

## I. Chronic Toxicity Summary

<i>Inhalation reference exposure level</i>	<b>100 <math>\mu\text{g}/\text{m}^3</math></b>
<i>Critical effect(s)</i>	Nasal cavity inflammation degeneration of the olfactory sensory epithelium in Fischer 344/N rats
<i>Hazard index target(s)</i>	Respiratory system; nervous system

## II. Physical and Chemical Properties (HSDB, 1995)

<i>Molecular formula</i>	$\text{C}_5\text{H}_8\text{O}_2$
<i>Molecular weight</i>	100.12
<i>Description</i>	Colorless liquid at room temp.; acrid, fruity odor
<i>Vapor Pressure</i>	40 mm Hg at 25.5°C
<i>Solubility</i>	Slightly soluble in water (1.5 g/100 g water at 30°C). Soluble in aromatic and chlorinated hydrocarbons
<i>Conversion factor</i>	4.09 $\mu\text{g}/\text{m}^3$ per ppb at 25°C

## III. Major Uses and Sources

Methyl methacrylate (MMA) readily polymerizes to form long-chain homopolymers and copolymers. MMA monomer is the most important ester of methacrylic acid commercially. Acrylic sheeting (Plexiglass), made by casting, moulding or extruding polymethyl or modified polymers, is the largest application for MMA. It is also used in the production of resins and surface coatings and as a water repellant for cement. In the medical field, MMA is used as a bone cement, a medicinal spray adhesive or nonirritant bandage solvent, and as a ceramic filler of cement by dentists. MMA is not known to occur in nature. MMA may enter the atmosphere or be released into wastewater or on land during its production, use in the manufacture of resins and plastics, transport or storage. Human exposures are primarily occupational by dermal and inhalation routes (HSDB, 1995).

#### IV. Effects of Human Exposures

A study of dental technicians working with acrylic plastics revealed that MMA is easily absorbed by the dermal route (Rajaniemi *et al.*, 1989). The metabolite, methacrylic acid, was found in urine of workers at the end of the work day. However, urinary levels of this metabolite were very low to undetectable the following morning, indicating that the half-life is short and that accumulation is unlikely.

Dermal contact with MMA cement in the medical and dental field has resulted in paresthesia of the fingers (Kassis *et al.*, 1984) and slower distal sensory conduction velocities from the digits (Seppalainen and Rajaniemi, 1984). In workers occupationally exposed to MMA for at least 5 years, chronic coughing was significantly greater when compared to a control group (Marez *et al.*, 1993). Mean atmospheric values of MMA at the two factories investigated were 18.5 and 21.6 ppm. Spirometric values at the beginning of the workshift were similar in both groups, but a mild airways obstruction appeared during the workshift. A Chinese study investigated two groups of workers exposed for up to 26 years to 11-33 and 100-200 mg/m<sup>3</sup> time-weighted average concentration of MMA (Lang *et al.*, 1986). A dose-dependent increase in the incidences of neurasthenia, laryngitis and hypotension was reported. Interactions of MMA with the endocrine system, resulting in altered levels of insulin, somatotrophic hormone and prolactin, were thought to be the cause of the adipogenicity observed in female but not male workers (Makarov *et al.*, 1981)

#### V. Effects of Animal Exposures

Up to 65% of a single dose of methyl[<sup>14</sup>C]methacrylate in rats is expired as <sup>14</sup>CO<sub>2</sub> in 2 hours, regardless of the route of administration (Bratt and Hathway, 1977). Up to 88% is expired as <sup>14</sup>CO<sub>2</sub> after 10 days. About half the remainder of the dose was excreted in the urine and the rest was retained in body tissues. Pulmonary excretion of unchanged MMA accounted for less than 1% of the dose. At least some of the urinary metabolites are excreted as mercapturic acids (Delbressine *et al.*, 1981). Metabolism of MMA is rapid. The methyl ester of the methacrylate acid ester is broken down in the body and the free acid enters the citric acid cycle after conjugation with coenzyme A (Bratt and Hathway, 1977).

In a six month inhalation study, 50 male rats (Charles River Sprague-Dawley)/group were exposed to 0 or 116 ppm MMA 8 hr/day, 5 days/week (Tansy *et al.*, 1976). No significant treatment-related changes were recorded regarding clinical chemistry and hematological parameters. Body and organ weights were not significantly changed following 6 month exposure to MMA. Histopathological examination of tissues was not performed in this report. The only observed effect was reduced body fat in rats exposed for 3 and 6 months to MMA. Mean body weights of exposed rats were not significantly lower than that of control rats at 3 and 6 months. Histopathological examination, published in a separate report (Tansy *et al.*, 1980), noted some focal lesions and epithelium denuded of cilia in the upper respiratory tract of rats exposed to 116

ppm of MMA for 6 months. However, only six animals from each exposure group (0 and 116 ppm MMA) were studied for airway damage and no attempt was made to quantitate the injury.

The most comprehensive long-term inhalation study performed with MMA to date is a 2 year study utilizing F344/N rats and B6C3F<sub>1</sub> mice (Chan *et al.*, 1988; NTP, 1986). Groups of 50 male rats were exposed to 0, 500 or 1000 ppm, groups of 50 female rats to 0, 250 or 500 ppm, and groups of 50 male and female mice to 0, 500 or 1000 ppm, 6 hr/day, 5 days/week. Body weights of the high dose rats were reduced 5-10% for more than 80 weeks of the study. Body weights of the low and high dose mice were reduced up to 19% for more than 20 weeks. Lesions in the nasal cavity of rats and mice in both treatment groups were significantly increased. In rats, inflammation and degeneration of the olfactory epithelium were observed and in mice, inflammation, hyperplasia, cytoplasmic inclusions in the respiratory epithelium and degeneration of the olfactory epithelium were observed. Necropsy and histologic examination revealed no other adverse effects in the major organ systems.

In a chronic oral toxicity study, MMA was added to drinking water of Wistar rats (25 rats/group/sex) in concentrations of 0, 6-7, 60-70 and 2000 ppm for two years (Borzelleca *et al.*, 1964). This fluid concentration was determined to be equivalent to about 0, 10, 100 and 3000 ppm in food. The same research group also administered MMA in gelatin capsules to beagle dogs for two years. The dogs received amounts equivalent to 0, 10, 100, and 1000-1500 ppm MMA in the diet. Mortality, body and organ weights, hematology and histopathology remained unaffected in both mammalian species following two year exposure to MMA.

No multi-generation MMA exposure studies have been performed. However, a well-conducted inhalation developmental toxicity study in rats was recently done (Solomon *et al.*, 1993). Exposure of concentrations of up to 2,028 ppm on days 6-15 of gestation resulted in no embryo or fetal toxicity or malformations, even at exposure levels that resulted in maternal toxicity.

## **VI. Derivation of Chronic Reference Exposure Level (REL)**

<i>Study</i>	Chan <i>et al.</i> , 1988; NTP, 1986
<i>Study population</i>	50 F344/N rats/group/sex and 50 B6C3F <sub>1</sub> mice/group/sex, 300 total for each species.
<i>Exposure method</i>	Discontinuous whole body inhalation exposure (0, 250 or 500 ppm for female rats; 0, 500 or 1000 ppm for mice and male rats)
<i>Critical effects</i>	Respiratory system (nasal cavity inflammation) and CNS/PNS (degeneration of the olfactory sensory epithelium).
<i>LOAEL</i>	250 ppm (1023 mg/m <sup>3</sup> ) in female rats; 500 ppm (2045 mg/m <sup>3</sup> ) in male rats and B6C3F <sub>1</sub> mice
<i>NOAEL</i>	Not observed
<i>Exposure continuity</i>	6 hr/day, 5 days/week
<i>Exposure duration</i>	2 years

<i>Average experimental exposure</i>	44.6 ppm for LOAEL group
<i>Human equivalent concentration</i>	7.2 ppm (gas with extrathoracic respiratory effects, RGDR = 0.16, BW = 229 g, MV = 0.17 L/min, SA = 15 cm <sup>2</sup> )
<i>LOAEL uncertainty factor</i>	10
<i>Subchronic uncertainty factor</i>	1
<i>Interspecies uncertainty factor</i>	3
<i>Intraspecies uncertainty factor</i>	10
<i>Cumulative uncertainty factor</i>	300
<i>Inhalation reference exposure level</i>	0.02 ppm (20 ppb, 0.1 mg/m <sup>3</sup> , 100 µg/m <sup>3</sup> )

Lifetime exposure of rats and mice to MMA resulted in chronic inflammatory responses in the nasal cavity of all treatment groups (Chan *et al.* 1988). Therefore, a NOAEL was not observed. The low and high doses for female rats were, respectively, 250 and 500 ppm while the low and high doses for male rats and male and female mice were, respectively, 500 and 1000 ppm. Therefore, the LOAEL is based on adverse effects seen in female rats. Nasal cavity lesions were similar in both male and female rats. In mice, the nasal cavity lesions consisted of inflammation, hyperplasia, cytoplasmic inclusions in the respiratory epithelium and degeneration of the olfactory epithelium. Other adverse effects near the LOAEL include decreased mean body weight (10-11%) in female rats of the 500 ppm group at a few time points during the second year of the study. Compared to controls, mean body weights of mice in both exposure groups were 10% lower or more during most of the second year.

An inhalation study by Tansy *et al.* (1980) noted evidence of nasal epithelial injury in rats exposed to 116 ppm MMA for 6 months. This LOAEL is lower than that reported by Chan *et al.* (1988). However, the Tansy *et al.* (1980) study did not characterize the nasal injury well, the number of rats investigated for this effect was low (6 rats/group), and the authors appeared uncertain whether this respiratory effect was a result of cellular injury or cellular immaturity due to a proliferative response. More data is needed before a NOAEL can be based on the results of Tansy *et al.* (1980).

One of the most recent studies of occupational exposure to MMA observed an increase in chronic coughing and mild airway obstruction (Marez *et al.*, 1993). A Chinese study of MMA occupational exposure reported a dose-dependent increase in laryngitis (Lang *et al.*, 1986). Therefore, adverse effects encountered by experimental animals and man following MMA inhalation appear consistent. Exposure to MMA by the oral route does not appear to be as toxic (Borzelleca *et al.*, 1964).

Weaknesses of the REL include limited data on human exposures and data on only two animal species exposed for extended periods. Study of a third non-rodent animal species would enhance the database for MMA chronic toxicity. A multi-generation study of MMA in an experimental animal species would also enhance the database.

## VII. References

- Borzelleca JF, Larson PS, Hennigar GR Jr, Huf EG, Crawford EM, and Smith BR Jr. 1964. Studies on the chronic oral toxicity of monomeric ethyl acrylate and methyl methacrylate. *Toxicol. Appl. Pharmacol.* 6:29-36.
- Bratt H, and Hathway DE. 1977. Fate of methyl methacrylate in rats. *Br. J. Cancer*, 36:114-119.
- Chan PC, Eustis SL, Huff JE, Haseman JK, and Ragan H. 1988. Two-year inhalation carcinogenesis studies of methyl methacrylate in rats and mice: Inflammation and degeneration of nasal epithelium. *Toxicology*, 52:237-252.
- Delbressine LP, Seutter-Berlage F, and Seutter E. 1981. Identification of urinary mercapturic acids formed from acrylate, methacrylate and crotonate in the rat. *Xenobiotica* 11(4):241-247.
- HSDB. 1995. Hazardous Substances Data Bank. National Library of Medicine, Bethesda, MD (CD-ROM version). Micromedex, Inc., Denver, CO (edition expires 11/31/95).
- Kassis V, Vedel P, and Darre E. 1984. Contact dermatitis to methyl methacrylate. *Contact Dermatitis*, 11:26.
- Lang Y-Y, Cai C-J, Wang Y-L, Xie Y-L, and Yang X-Y. 1986. Observations on the effects of exposure to methyl methacrylate on worker's health. *Chin. J. Prev. Med.* 20:344-347 (in Chinese).
- Makarov IA, Makarenko KI, and Desyatnikova NV. 1981. On the adipogenic effect of some industrial poisons. *Gig. Tr. Prof. Zabol.* 12:29-31 (in Russian).
- Marez T, Edme JL, Boulenguez C, Shirali P, and Haguenoer JM. 1993. Bronchial symptoms and respiratory function in workers exposed to methylmethacrylate. *Br. J. Ind. Med.* 50:894-897.
- NTP. 1986. National Toxicology Program. Toxicology and carcinogenesis studies of methyl methacrylate in F344/N rats and B6C3F<sub>1</sub> mice. Technical Report Series NTP-TR-314.
- Rajaniemi R, Pfaffli P, and Savolainen H. 1989. Percutaneous absorption of methyl methacrylate by dental technicians. *Br. J. Ind. Med.* 46:356-357.
- Seppalainen A, and Rajaniemi R. 1984. Local neurotoxicity of methyl methacrylate among dental technicians. *Am. J. Ind. Med.* 5:471-.
- Solomon HM, McLaughlin JE, Swenson RE, Hagan JV, Wanner FJ, O'Hara GP, and Krivanek ND. 1993. Methyl methacrylate: Inhalation developmental toxicity study in rats. *Teratology*, 48:115-125.

Tansy MF, Kendall FM, Benhayem S, Hohenleitner FJ, Landin WE, and Gold M. 1976. Chronic biological effects of methyl methacrylate vapor. I. Body and tissue weights, blood chemistries, and intestinal transit in the rat. Environ. Res., 11:66-77.

Tansy MF, Hohenleitner FJ, White DK, Oberly R, Landin WE, and Kendall FM. 1980. Chronic biological effects of methyl methacrylate vapor. III. Histopathology, blood chemistries, and hepatic and ciliary function in the rat. Environ. Res. 21:117-125.

CHRONIC TOXICITY SUMMARY

**METHYL t-BUTYL ETHER**

(MTBE; 2-methoxy-2-methylpropane; tert-butyl methyl ether;  
methyl 1,1dimethyl ether)

**CAS Registry Number: 1634-04-4**

**I. Chronic Toxicity Summary**

<i>Inhalation reference exposure level</i>	<b>3 mg/m<sup>3</sup> (0.8 ppm)</b> (U.S. EPA RfC) This document summarizes the evaluation of non-cancer health effects by U.S. EPA for the RfC
<i>Critical effect(s)</i>	Nephrotoxicity, prostration, periocular swelling in Fisher 344 rats
<i>Hazard index target(s)</i>	Kidney; eyes; alimentary system

**II. Physical and Chemical Properties (HSDB, 1994)**

<i>Molecular formula</i>	C <sub>5</sub> H <sub>12</sub> O
<i>Molecular weight</i>	88.15 g/mol
<i>Description</i>	Colorless liquid
<i>Specific gravity</i>	0.7405 @ 20°C
<i>Boiling point</i>	55.2°C @ 760 mm Hg
<i>Vapor pressure</i>	245 mm Hg @ 20°C
<i>Solubility</i>	Soluble in alcohol, ether, and 5% soluble in water
<i>Conversion factor</i>	1 ppm = 3.61 mg/m <sup>3</sup> @ 25° C; 3.67 mg/m <sup>3</sup> @ 20° C

**III. Major Uses or Sources**

Methyl t-butyl ether (MTBE) is used as a gasoline additive to improve octane ratings and reduce emissions of some pollutants, in industry to improve miscibility of solvents, and in clinical medicine to dissolve cholesterol gall stones (Yoshikawa *et al.*, 1994).

**IV. Effects of Human Exposure**

No human chronic toxicity or chronic epidemiology information for MTBE was found.

## V. Effects of Animal Exposure

Male and female rats (50/sex/group) were exposed by inhalation for 6 hours/day, 5 days/week to mean concentrations of 0, 403, 3023, or 7977 ppm (0, 1453, 10,900, or 28,760 mg/m<sup>3</sup>) MTBE for 24 months (Chun *et al.*, 1992). Clinical signs, hematology, body weights and food consumption were monitored. Necropsy included measurements of organ weights and histopathology. Corticosterone levels were measured on 10 animals prior to sacrifice. Serum enzymes were not monitored. The NOAEL for several endpoints, including non-alpha-2μ-globulin induced nephrotoxicity, increased relative liver and kidney weights and prostration in females, and periocular swelling in both sexes was 403 ppm (1453 mg/m<sup>3</sup>).

Mice were exposed for 6 hours/day, 5 days/week for 18 months to MTBE concentrations of 0, 402, 3014, or 7973 ppm (0, 111, 835, or 2208 mg/m<sup>3</sup>) (Burleigh-Flayer *et al.*, 1992). The mice exposed to the highest concentration (7973 ppm) all exhibited ataxia. Prostration was also noted in 8 of 50 animals in this group. Liver weights were elevated in a concentration-dependent manner in the female mice but this change was not significant at the lowest concentration (402 ppm). Kidney weights were elevated in the female mice exposed to 7973 ppm. At the highest concentration, a significant increase in hepatocellular hypertrophy and adrenal gland weight was detected in the male mice. Spleen weights were increased in the females exposed to the highest concentration.

Tests for histopathology in the respiratory tract, plasma corticosterone levels, motor activity and neurobehavioral endpoints were performed in rats exposed to MTBE at concentrations of 0, 797, 3920, or 8043 ppm (0, 2877, 14151, or 29035 mg/m<sup>3</sup>), 6 hours/day, 5 days/week for 13 weeks (Dodd and Kintigh, 1989). Of these endpoints, the most significant finding was an elevation in plasma corticosterone in the high dose group. This finding was consistent with the elevated adrenal weights reported by Burleigh-Flayer *et al.* (1992). A clear dose-response for neurotoxic effects in these rats was not established.

Biles *et al.* (1987) reported a NOAEL of 300 ppm (1083 mg/m<sup>3</sup>) MTBE for decreased pup viability in rats exposed for 6 hours/day, 5 days/week for a total of 16 weeks. Animals exposed to 1240 ppm (4470 mg/m<sup>3</sup>) or 2860 ppm (10,311 mg/m<sup>3</sup>) MTBE exhibited slightly decreased pup survival.

Neeper-Bradley (1991) exposed rats to 0, 402, 3019, or 8007 ppm (0, 111, 836, and 2218 mg/m<sup>3</sup>) MTBE over 2 generations. Exposures were for 6 hours/day, 5 days/week during the prebreeding period, and for 7 hours/day, 5 days/week during gestation and lactation. Parental effects of MTBE exposure were observed, including ataxia, blepharospasm, lack of startle reflex, and increased relative liver weights (F1 generation only). There were no histological changes in the organs from either parental generation. Reduced body weights were observed in the F1 and F2 pups at the 3019 and 8007 ppm concentrations. Reduced survivability to postnatal day 4 was observed in the 8007 ppm group. No adverse effects were noted at the 403 ppm (111 mg/m<sup>3</sup>) concentration.

In a developmental and reproductive toxicity study, Conaway and associates (1985) found no significant increases in maternal or fetal toxicity, nor in pregnancy rates or in any gross toxicologic parameter tested with pregnant rats or mice exposed during gestation to concentrations of MTBE up to 3300 ppm (11,897 mg/m<sup>3</sup>).

Maternal toxicity, in the form of hypoactivity and ataxia was observed in pregnant mice exposed during gestation to 4076 ppm (14,690 mg/m<sup>3</sup>) MTBE (Bushy Run Research Center, 1989a). Significant reductions in food intake and body weight gain were observed in dams exposed to 8153 ppm (29,390 mg/m<sup>3</sup>). Fetal body weight was significantly reduced in the 4076 ppm group, and there were significant increases in the incidences of skeletal variations and unossified phalanges in the 4076 and 8153 ppm groups. Pregnant rabbits exposed to similar concentrations during gestation showed no significant maternal or fetal toxicity or developmental toxicity up to a concentration of 8021 ppm (28,918 mg/m<sup>3</sup>) (Bushy Run Research Center, 1989b).

## VI. Derivation of U.S. EPA RfC

<i>Study</i>	Chun <i>et al.</i> , 1992 (U.S. EPA, 1995)
<i>Study population</i>	Male and female rats (50 per sex/group)
<i>Exposure method</i>	Discontinuous whole-body inhalation exposures (0, 403, 3023, or 7977 ppm)
<i>Critical effects</i>	Nephrotoxicity, increased liver and kidney weight, prostration and periorcular swelling
<i>LOAEL</i>	3023 ppm
<i>NOAEL</i>	403 ppm
<i>Exposure continuity</i>	6 hours per day, 5 days per week
<i>Exposure duration</i>	24 months
<i>Average experimental exposure</i>	72 ppm for the NOAEL group
<i>Human equivalent concentration</i>	72 ppm for the NOAEL group (gas with systemic effects, based on RGDR = 1.0 using default assumption that lambda (a) = lambda (h))
<i>LOAEL uncertainty factor</i>	1
<i>Subchronic uncertainty factor</i>	1
<i>Interspecies uncertainty factor</i>	3
<i>Intraspecies uncertainty factor</i>	10
<i>Modifying factors</i>	3 (lack of reproductive and developmental toxicity data)
<i>Cumulative uncertainty factor</i>	100
<i>Inhalation reference exposure level</i>	0.8 ppm (800 ppb, 3 mg/m <sup>3</sup> , 3000 µg/m <sup>3</sup> )

The major strengths of the REL are the use of a comprehensive, long-term multiple dose study with large sample sizes and the observation of a NOAEL. The major uncertainty is the lack of human data.

## VII. References

- Biles RW, Schroeder RE, and Holdsworth CE. 1987. Methyl tertiary butyl ether inhalation in rats: A single generation study. *Toxicol. Indust. Health.* 3:519-534.
- Burleigh-Flayer HD, Chun JS, and Kintigh WJ. 1992. Methyl tertiary butyl ether: Vapor inhalation oncogenicity study in CD- 1 mice (unpublished material). Prepared for the MTBE Committee by Bushy Run Research Center, Union Carbide Chemicals and Plastics Company, Inc. Docket No. OPTS-42098.
- Bushy Run Research Center, Union Carbide Chemicals and Plastics Company, In. 1989a. Developmental toxicity study of inhaled methyl tertiary butyl ether in CD- 1 mice (final report). TSCATS/403186. EPA/OTS No. FYI-OTS-0889-0689. [cited in U.S. EPA, 1995].
- Bushy Run Research Center, Union Carbide Chemicals and Plastics Company, In. 1989b. Developmental toxicity study of inhaled methyl tertiary butyl ether in New Zealand White rabbits (final report). TSCATS/403186. EPA/OTS No. FYI-OTS-0889-0689. [cited in U.S. EPA, 1995].
- Chun JS, Burleigh-Flayer HD, and Kintigh WJ. 1992. Methyl tertiary butyl ether: Vapor inhalation oncogenicity study in Fischer 344 rats (unpublished material). Prepared for the MTBE committee by Bushy Run Research Center, Union Carbide Chemicals and Plastics Company, Inc., Docket No. OPTS-42098.
- Conaway CC, Schroeder RE, and Snyder NK. 1985. Teratology evaluation of methyl tertiary butyl ether in rats and mice. *J. Toxicol. Environ. Health.* 16(6):797-809.
- Dodd DE, and Kintigh WJ. 1989. Methyl tertiary butyl ether (MTBE): Repeated (13 week) vapor inhalation study in rats with neurotoxicity evaluation (unpublished material). Prepared for the MTBE Committee by Bushy Run Research Center, Union Carbide Chemicals and Plastics Company, Inc., TSCATS 403187. EPA/OTS No. FYI-OTS-08890689.
- HSDB. 1994. Hazardous Substance Databank. National Library of Medicine, Bethesda, MD (CD-ROM version). Micromedex, Inc., Denver, CO (edition expired 10/31/94).
- Neeper-Bradley TL. 1991. Two-generation reproduction study of inhaled methyl tertbutyl ether in CD Sprague-Dawley rats (unpublished material). Bushy Run Research Center, Union Carbide Chemicals and Plastics Company, Inc.
- U.S.EPA. 1995. U.S. Environmental Protection Agency. Integrated Risk Information System (IRIS) database. Reference concentration (RfC) for methyl t-butyl ether (MTBE).
- Yoshikawa M, Arashidani K, Katoh T, Kawamoto T, and Kodama Y. 1994. Pulmonary elimination of methyl tertiary butyl ether after intraperitoneal administration in mice. *Arch. Toxicol.* 68:517-519.

CHRONIC TOXICITY SUMMARY

**METHYLENE CHLORIDE**

(dichloromethane, methylene dichloride)

**CAS Registry Number: 75-9-2**

**I. Chronic Toxicity Summary**

<i>Inhalation reference exposure level</i>	<b>300 µg/m<sup>3</sup></b>
<i>Critical effect(s)</i>	Carboxyhemoglobin formation above 2% in human workers
<i>Hazard index target(s)</i>	Circulatory system; nervous system

**II. Physical and Chemical Properties** (HSDB, 1995, except as noted)

<i>Description</i>	Colorless liquid
<i>Molecular formula</i>	CH <sub>2</sub> Cl <sub>2</sub>
<i>Molecular weight</i>	84.93
<i>Specific gravity</i>	1.32 @ 20° C (ACGIH, 1991)
<i>Boiling point</i>	39.75° C
<i>Vapor pressure</i>	400 mm Hg @ 24.1° C
<i>Solubility</i>	Miscible with most organic solvents, slightly soluble in water (ACGIH, 1991)
<i>Conversion factor</i>	1 ppm = 3.47 mg/m <sup>3</sup> @ 25° C

**III. Major Uses and Sources**

Methylene chloride (MC) is used in paint and varnish remover, in aerosols as a cosolvent or vapor pressure depressant and in solvent degreasing and metal cleaning. It is also used in plastics processing and in extraction of fats and oils from food products (HSDB, 1995).

**IV. Effects of Human Exposure**

Effects of a controlled 2-hour inhalation exposure to MC included CNS depression at concentrations of 1000 ppm (3500 mg/m<sup>3</sup>) or more and increased blood carboxyhemoglobin (COHb) content at lower concentrations (500 ppm) due to metabolism of MC to carbon monoxide (Stewart *et al.*, 1972). High levels of COHb can be found in the blood hours after exposure to methylene chloride, due to its partitioning into fat and slow release into circulation with subsequent metabolism, leading to formation of carbon monoxide (Engstrom and

Bjurstrom, 1977). In situations of chronic exposure, carbon monoxide toxicity is also of concern. Barrowcliff (1978) documented the case of an adult male who developed an unsteady gait, a peculiar dysarthria and a loss of memory. The man had worked with 15-50 liters of methylene chloride daily for 3 years in a poorly ventilated room while cleansing road materials. No natural disease could be found to explain his conditions and the effects were attributed to chronic carbon monoxide poisoning.

Twelve women volunteer subjects were exposed to 0, 300, or 800 ppm methylene chloride for 4 hours (Fodor and Winneke, 1971). Neurobehavioral vigilance was measured by auditory discrimination of intensity of certain sound pulses against a background of continuous white noise. A significant interactive effect between methylene chloride concentration and duration of exposure using 2-way ANOVA ( $p < 0.01$ ) was found.

Human erythrocytes enzymatically convert methylene chloride to formaldehyde in cell-culture experiments (Hallier *et al.*, 1994).

A subacute controlled exposure of eleven resting non-smokers to methylene chloride was conducted by DiVincenzo and Kaplan (1981a). The eleven subjects were exposed to 50, 100, 150, or 200 ppm methylene chloride for 7.5 hours on 5 consecutive days. Exposure to all concentrations led to dose-dependent elevation in COHb concentrations in the blood and elevated exhaled CO. The peak blood COHb saturations were 1.9, 3.4, 5.3, and 6.8% respectively for the 50, 100, 150, and 200 ppm groups. Divincenzo and Kaplan (1981a) also measured COHb percentage in the blood of workers exposed to a mean concentration of methylene chloride of 40 ppm (range = 0 to 250 ppm), compared with control workers exposed to 0 ppm methylene chloride for an 8-hour day. The 19 workers exposed to methylene chloride had mean blood COHb concentrations of 2.3% in the morning and 3.9% at the end of the workshift. Controls (8 subjects) had significantly lower mean blood COHb concentrations of 0.8% in the AM and 1.3% in the PM compared with the exposed workers. The length of employment of the exposed workers was not given.

A companion study by DiVincenzo and Kaplan (1981b) showed that smoking and methylene chloride exposure result in an additive effect on COHb saturation compared with saturation in non-smokers. Similarly, light, moderate or heavy exercise workloads resulted in higher COHb saturation.

Soden *et al.* (1996) showed a dose-response increase in carboxyhemoglobin levels in non-smokers with increasing methylene chloride exposure in workers involved in triacetate fiber production. Carboxyhemoglobin levels ranged from 1.77% to 4% from exposures ranging from 6.45 to 89.69 ppm, respectively. The number of employees in the study was not reported.

Although animal studies have shown COHb-induced cardiovascular effects following MC exposure (Aviado *et al.*, 1977), no data exist on this outcome in humans. However, studies of men with coronary artery disease and exercise-induced angina report a decrease in time to onset of exercise-induced angina following exposure to carbon monoxide (CO) at concentrations sufficient to result in blood COHb levels of about 2% (Kleinman *et al.*, 1989; Allred *et al.*,

1989). From a physiologically based pharmacokinetic model of MC and CO it was estimated that a 1-hour exposure to 340 ppm (1200 mg/m<sup>3</sup>) MC at a ventilation rate of 9 liters/min would result in a peak blood COHb level of 2% (Andersen *et al.*, 1991; Reitz, 1994). The California Ambient Air Quality Standard for CO is based on a blood COHb level of 2% (CARB, 1982).

An epidemiological study of 751 male workers in the Eastman Kodak company exposed to daily 8-hour time-weighted average concentrations of 30-125 ppm methylene chloride for up to 30 years was conducted by Friedlander and associates (1978). A control group of workers in production but not exposed to methylene chloride was used together with New York state cause and age-specific mortality rates. The follow-up period for these workers was 13 years, with 97% success. The studies did not indicate any increase in risk of death from circulatory disease, cancer, or other causes due to methylene chloride exposure.

A study of female pharmaceutical workers in eight different factories exposed to a variety of organic solvents indicated that solvent exposure, and particularly methylene chloride exposure, resulted in an increase in spontaneous abortions (Taskinen *et al.*, 1986). In all, 1795 pregnancies were followed, with 142 spontaneous abortions occurring. The odds ratio for methylene chloride exposure was 1.0 to 5.7 (average = 2.3;  $p < 0.06$ ). There was a significant effect of exposure to 4 or more solvents, compared with age-matched controls ( $p < 0.05$ ). The concentrations of MC were not reported in the study.

The U.S. Occupational Safety and Health Administration reduced its permissible exposure limits for MC from 500 ppm to 25 ppm in 1997 (U.S. CFR, 1997).

## **V. Effects of Animal Exposure**

Nitschke *et al.* (1988) found that a 2-year exposure to 0, 50, 200, or 500 ppm MC for 6 hours/day, 5 days/week resulted in significant histopathologic lesions in the livers of rats exposed to 500 ppm. No significant adverse effects were observed at 200 ppm or lower. The predominant hepatocellular lesion was fatty vacuolization of hepatocytes.

A continuous exposure of mice (16 per group) to 100 ppm MC for 1, 2, 3, 4 or 10 weeks resulted in significant elevation in liver triglycerides beginning at 2 weeks and lasting throughout the 10-week period (Weinstein and Diamond, 1972). Liver/body weight ratios were unaffected at any time point. After 1 week, small fat droplets were apparent in centrilobular hepatocytes and a decrease in hepatic glycogen was also noted. Necrosis was not observed during the 10-week period, but fat droplet size increased and glycogen depletion persisted.

Elevated carboxyhemoglobin levels and liver histological changes were observed in rats and hamsters exposed to 500, 1500, or 3500 ppm methylene chloride 6 hours/day, 5 days/week (excluding holidays), for 2 years (Burek *et al.*, 1984). The groups consisted of 129 rats per sex per concentration, and 107 to 109 hamsters per sex per concentration.

Monkeys were observed to be more susceptible subjects for methylene chloride induced COHb than dogs upon 14-week subchronic continuous exposure to 25 or 100 ppm (Haun *et al.*, 1972). At 25 ppm, approximately 1.5% COHb was reached in the 4 monkeys, compared to approximately 0.5% in 16 dogs. Monkeys exposed to 100 ppm MC had COHb levels of approximately 4% compared with 2% in the dogs.

Oral ethanol pretreatment in rats has been shown to suppress the COHb formation characteristic of methylene chloride exposure through inhibition of biotransformation of methylene chloride (Glatzel *et al.*, 1987).

Gerbils (10/sex per group; 60 controls) exposed continuously to MC concentrations of 210, 350, or 700 ppm for a period of 3 months, with a 4-month follow-up period showed irreversible brain cellular and biochemical changes (Rosengren *et al.*, 1986). A high mortality rate (19/20) was observed in the 700 ppm group, and this exposure was terminated after 7 weeks. The gerbils exposed to 350 ppm also had a high mortality rate (9/20) and this exposure was terminated after 10 weeks. The gerbils exposed to 210 ppm had no premature mortality and the exposure continued for the full 3 months. Four months after termination of exposure, the animals in the 350 and 210 ppm groups had significantly decreased brain DNA content in the hippocampus. The 350 ppm group exhibited elevated astroglial proteins in the frontal and sensory motor cerebral cortex, consistent with astrogliosis in these regions. In addition, the gerbils exposed to 350 ppm MC had significantly decreased DNA in the cerebellar hemispheres. Complimentary studies by these investigators showed that the formation of carboxyhemoglobin did not increase in gerbils between the 210 and 350 ppm exposures, indicating that the metabolism of MC to CO is saturable at concentrations below those in the study. On the other hand, the neurotoxic brain biochemical alterations were significantly greater in gerbils exposed to 350 ppm as compared with the 210 ppm group, implying that carboxyhemoglobin induced cerebral hypoxia is not the major cause of MC-induced neurotoxicity in the brain.

Rats (50 per sex per group) were exposed to 0, 1000, 2000, or 4000 ppm methylene chloride 6 hours/day, 5 days/week for 102 weeks (NTP, 1986). Both sexes exhibited hemosiderin pigmentation in the liver in a dose-dependent fashion, beginning with the 1000 ppm concentration. Squamous metaplasia of the nasal cavity was observed in female rats, and thyroid C-cell hyperplasia was observed in males exposed to 2000 ppm or greater. Kidney tubule degeneration (not otherwise specified) was increased at all exposure levels.

Mice (50 per sex per group) exposed to 0, 2000, or 4000 ppm methylene chloride 6 hours/day, 5 days/week for 102 weeks showed increased incidence of liver cytologic degeneration and splenic atrophy at 4000 ppm (males) (NTP, 1986). Male and female mice also had an increased incidence of kidney tubule casts (not otherwise specified) at 2000 ppm or greater, and significant testicular atrophy was observed in males at 4000 ppm. Female mice showed cytologic degeneration in the liver at 2000 ppm or greater, and ovarian atrophy at 2000 ppm or greater.

A six month exposure to 5000 ppm MC of 8 guinea pigs for 7 hours/day, 5 days/week resulted in 3 deaths; 2 showed moderate centrilobular fatty degeneration of the liver and extensive pneumonia observed at necropsy (Heppel *et al.*, 1944). None of the 14 control animals died.

Food consumption and body weight were lower in the exposed guinea pigs, compared with control pigs. One out of 12 rats died at this concentration, and the liver histology in this animal revealed multiple thrombi in renal vessels, associated with marked cortical infarction. By comparison, dogs and rabbits showed no signs of illness, nor were blood pressure or hematological values altered at the 5000 ppm concentration. At 10,000 ppm, 2 of 4 dogs showed moderate centrilobular congestion, narrowing of liver cell cords, and slight to moderate fatty degeneration. One of 2 monkeys revealed disseminated tuberculosis lesions, but no other histological alterations. Four out of 6 guinea pigs had moderate fatty degeneration of the liver at this concentration.

The offspring of rats (10 dams per group) exposed during gestation to 0 or 4500 ppm methylene chloride exhibited altered rates of behavioral habituation to novel environments (Bornschein *et al.*, 1980). This effect was observed beginning at 10 days of age but was still demonstrable in rats 150 days old. The authors concluded that elevated maternal COHb could have been a contributing factor in the developmental impairment.

In a study of the effects of methylene chloride on estrous cycle and serum prolactin, groups of 15 female rats were exposed to 0 or 3500 ppm for 6 hours/day for 15 to 19 consecutive days (Breslin and Landry, 1986). Males (15 per group) were exposed for 5 hours/day for 5 consecutive days. Female rats exhibited decreased body weight and an increase in the estrous cycle duration and in serum prolactin. Males did not show any significant effects on serum prolactin from methylene chloride exposure.

Pregnant mice and rats were exposed to 0, or 1250 ppm MC 7 hours/day, on days 6 through 15 of gestation (Schwetz *et al.*, 1975). Significantly elevated absolute liver weights were seen in maternal animals from both species. In addition, significantly increased incidences of delayed ossification of the sternebrae were seen in both species, compared to controls.

Methylene chloride exposure of 4500 ppm to female rats before or during gestation resulted in elevated maternal liver weights and decreased birth weights of the offspring, but no terata or skeletal/soft tissue anomalies (Hardin and Manson, 1980).

A 2-generation reproduction test was conducted by Dow Chemical Company (Nitschke *et al.*, 1985) which showed no significant reproductive or developmental effects in rats exposed to 0, 100, 500, or 1500 ppm MC 6 hours/day, 5 days/week, for 14 weeks. The exposure conditions were identical for the F0 and F1 generations.

## VI. Derivation of Chronic Reference Exposure Level (REL)

<i>Study</i>	DiVincenzo and Kaplan (1981a)
<i>Study population</i>	19 workers, 8 controls
<i>Exposure method</i>	Occupational inhalation exposure
<i>Critical effects</i>	Significantly elevated carboxyhemoglobin levels (> 2%)
<i>LOAEL</i>	33 ppm (range = 0 - 250 ppm); controls = 0 ppm
<i>NOAEL</i>	Not established
<i>Exposure continuity</i>	8 hours/day, 5 days/week
<i>Exposure duration</i>	Length of employment unspecified
<i>Average occupational exposure</i>	8 ppm for LOAEL group
<i>LOAEL uncertainty factor</i>	10
<i>Subchronic uncertainty factor</i>	1 (see following text for explanation)
<i>Interspecies uncertainty factor</i>	1
<i>Intraspecies uncertainty factor</i>	10
<i>Cumulative uncertainty factor</i>	100
<i>Inhalation reference exposure level</i>	0.08 ppm (80 pbb; 0.3 mg/m <sup>3</sup> ; 300 µg/m <sup>3</sup> )

Workers were exposed to average measured concentrations of 40 ppm during the workday, and the personal monitors on 3 of the subjects indicated a 8-hour time-weighted average of 33 ppm over a 2-week period. The average COHb levels were 3.9% at the end of the workshift. Elevated carboxyhemoglobin concentrations of above 2% are considered to aggravate angina in some individuals (CARB, 1982). In effect, 2% COHb can be considered a NOAEL for aggravation of angina. Therefore, the 33 ppm concentration was considered a LOAEL for the formation of greater than 2% COHb. The duration of the employment period was not specified. However, in the DiVincenzo and Kaplan (1981a) study, the levels of COHb did not appear to increase over a period of 5 days in experimental exposures using volunteers, therefore an uncertainty factor for subchronic exposure was not necessary. A number of factors contribute to the uncertainty in determining the degree of sensitivity to methylene chloride, including activity level, metabolic enzyme activity, age, and background COHb status (e.g. from smoking, etc.).

The subchronic study by Haun *et al.* (1972) with monkeys reported a NOAEL of 25 ppm and a LOAEL of 100 ppm for 2% COHb formation following a 14-week exposure. These results are consistent with the LOAEL reported in the DiVincenzo and Kaplan study. However, the human occupational study likely contains less uncertainty, since the toxicokinetics of the effect, including rate of formation of CO and thus COHb is metabolism-dependent, resulting in considerable potential interspecies differences.

The major strength of the REL is the use of human health effects data. The major uncertainties are the lack of a NOAEL observation, the difficulty in estimating exposures, and the discontinuous and variable nature of the exposures.

## VII. References

- Allred EN, Bleecker ER, Chaitman BR, Dahms TE, Gottlieb SO, Hackney JD, Hayes D, Pagano M, Selvester RH, Walden SM, and Warren J. 1989. Acute effects of carbon monoxide exposure on individuals with coronary artery disease. Health Effects Research Institute (HEI) Research Report No. 25., Cambridge, MA., HEI.
- ACGIH. 1991. American Conference of Governmental Industrial Hygienists. Documentation of Threshold Limit Values and Biological Exposure Indices. 6th edition ACGIH, Cincinnati, OH.
- Andersen ME, Clewell HJ III, Gargas ML, MacNaughton MG, Reitz RH, Nolan RJ, and McKenna MJ. 1991. Physiologically based pharmacokinetic modeling with dichloromethane, its metabolite, carbon monoxide, and blood carboxyhemoglobin in rats and humans. *Toxicol. Appl. Pharmacol.* 108:14-27.
- Aviado DM, Zakhari S, Watanabe T. 1977. Methylene Chloride. In Goldberg, L. (ed.) *Non-Fluorinated Propellants and Solvents for Aerosols*, Cleveland, OH: CRC Press, pp.19-45.
- Barrowcliff DF. 1978. Chronic carbon monoxide poisoning caused by methylene chloride paintstripper. *Med. Sci. Law* 18(4):238.
- Bornschein RL, Hastings L, and Manson JM. 1980. Behavioral toxicity in the offspring of rats following maternal exposure to dichloromethane. *Toxicol. Appl. Pharmacol.* 52:29-37.
- Breslin WJ, and Landry TD. 1986. Methylene chloride: Effects on estrous cycling and serum prolactin in Sprague-Dawley rats. Unpublished study by Dow Chemical U.S.A., Midland, MI 48674.
- Burek JD, Nitschke KD, Bell TJ, Wackerle DL, Childs RC, Beyer JE, Dittenber DA, Rampy LW, and McKenna MJ. 1984. Methylene chloride: A two-year inhalation toxicity and oncogenicity study in rats and hamsters. *Fundam. Appl. Toxicol.* 4:30-47.
- DiVincenzo GD, and Kaplan CJ. 1981a. Uptake, metabolism, and elimination of methylene chloride vapor by humans. *Toxicol. Appl. Pharmacol.* 59:130-140.
- DiVincenzo GD, and Kaplan CJ. 1981b. Effect of exercise or smoking on the uptake, metabolism, and excretion of methylene chloride vapor. *Toxicol. Appl. Pharmacol.* 59:141-148.
- Engstrom J, and Bjurstrom R. 1977. Exposure to methylene chloride: content in subcutaneous adipose tissue. *Scand. J. Work Environ. Health* 3:215-224.
- Fodor GG, and Winneke H. 1971. Nervous system disturbances in men and animals experimentally exposed to industrial solvent vapors. *Proc. 2nd Intl. Clean Air Congr.* Academic Press, NY. pg. 238-243.

Friedlander BR, Hearne T, and Hall S. 1978. Epidemiologic investigation of employees chronically exposed to methylene chloride. J. Occup. Med. 20:657-666.

Glatzel W, Tietze K, Gutewort R, and Pankow D. 1987. Interaction of dichloromethane and ethanol in rats: Toxicokinetics and nerve conduction velocity. Alcoholism: Clin. Exp. Res. 11(5):450-452.

Hallier E, Schroder KR, Asmuth K, Dommermuth A, Aust B, Goerens HW. 1994. Metabolism of dichloromethane (methylene chloride) to formaldehyde in human erythrocytes: influence of polymorphism of glutathione transferase theta (GST T1-1). Arch. Toxicol. 68:423-427.

Haun CC, Vernot EH, and Darmer KI. 1972. Continuous animal exposure to low levels of dichloromethane. Aerospace Medical Research Laboratories, Wright-Patterson Air Force Base, Dayton, OH. AMRL-TR-72-130.

HSDB. 1995. Hazardous Substance Data Bank. National Library of Medicine, Bethesda, Maryland (CD-ROM Version). Micromedex Inc., Denver, Colorado (Edition expires 1/31/95).

Heppel LA, Neal PA, Perrin TL, Orr ML, and Porterfield VT. 1944. Toxicology of dichloromethane (methylene chloride), I: Studies on effects of daily inhalation. J. Ind. Hyg. Toxicol. 26(1):8-16.

Kleinman MT, Davidson DM, Vandigraff RB, Caiozzo VJ, and Whittenberger JL. 1989. Effects of short-term exposure to carbon monoxide in subjects with coronary artery disease. Arch. Env. Health 44(6):361-369.

NTP. 1986. National Toxicology Program. Toxicology and carcinogenesis studies of dichloromethane (methylene chloride) in F344/N rats and B6C3F1 mice. U.S. Dept. of Health and Human Services. NIH Publication 86-2562. Research Triangle Park, NC.

Nitschke KD, Burek JD, Bell TJ, Kociba RJ, Rampey LW, and McKenna MJ. 1988. Methylene chloride: A 2-year inhalation toxicity and oncogenicity study in rats. Fundam. Appl. Toxicol. 11:48-59.

Reitz RH. 1994. Personal communication. August 5, 1994.

Rosengren LE, Kjellstrand P, Aurell A, and Haglid KG. 1986. Irreversible effects of dichloromethane on the brain after long term exposure: a quantitative study of DNA and the glial cell marker proteins S-100 and GFA. Br. J. Ind. Med. 43:291-299.

Soden KJ, Marras G, and Amsel J. 1996. Carboxyhemoglobin levels in methylene chloride-exposed employees. J. Occup. Environ. Med. 38(4):367-371.

CARB. 1982. California Air Resources Board. California Ambient Air Quality Standards for Carbon Monoxide (Sea Level). Technical Support Document, State of California Air Resources Board, Technical Support Division.

Stewart RD, Fisher TN, Hosko MJ, Peterson JE, Baretta ED, and Dodd HC. 1972. Experimental human exposure to methylene chloride. Arch. Env. Health 25(5):342-348.

Taskinen H, Lindbohm ML, and Hemminki K. 1986. Spontaneous abortions among women working in the pharmaceutical industry. Br. J. Ind. Med. 43:199-205.

U.S. Code of Federal Regulations (29 CFR). Final Rule for Occupational Exposure to Methylene Chloride. Federal Register 62(7):1494.

Weinstein RS, and Diamond SS. 1972. Hepatotoxicity of dichloromethane (methylene chloride) with continuous inhalation exposure at a low dose level. Proceedings of the 3rd annual conference on environmental toxicology, 25, 26, and 27 October, 1972. Aerospace Medical Research Laboratory, Aerospace Medical Division, Air Force Systems Command, Wright-Patterson Air Force Base, OH.

CHRONIC TOXICITY SUMMARY

## 4,4'-METHYLENE DIANILINE

(MDA; 4,4'-diaminodiphenylmethane; 4,4'-diphenylmethanedianiline; DAPM; dianilinmethane)

CAS Registry Number: 101-77-9

### I. Chronic Toxicity Summary

<i>Inhalation reference exposure level</i>	<b>20 µg/m<sup>3</sup></b>
<i>Critical effect(s)</i>	Ocular toxicity to the retinas of guinea pigs
<i>Hazard index target(s)</i>	Eyes; alimentary system

### II. Chemical Property Summary (HSDB, 1995)

<i>Molecular formula</i>	C <sub>13</sub> H <sub>14</sub> N <sub>2</sub>
<i>Molecular weight</i>	198.3 g/mol
<i>Description</i>	Colorless to pale yellow flakes; tan
<i>Vapor pressure</i>	1 mm Hg @ 197°C
<i>Solubility</i>	Soluble in alcohol, benzene, ether; 273g/100g acetone; 0.1g/100g water @ 25°C
<i>Conversion factor</i>	8.1µg/m <sup>3</sup> per ppb at 25°C

### III. Major Uses and Sources

4,4'-Methylene dianiline (MDA) is synthesized by the reaction of aniline with formaldehyde. MDA's major uses are as a chemical intermediate in the synthesis of certain isocyanates and polyurethane polymers, as a corrosion inhibitor, in the preparation of azo dyes, as a rubber preservative, and in the curing of epoxy resins and neoprene (HSDB, 1995; ACGIH, 1992).

### IV. Effects of Human Exposure

Several cases of human exposure to MDA have clearly identified the compound as a hepatotoxicant which produces a condition resembling viral hepatitis (Kopelman *et al.*, 1966; McGill and Motto, 1974; Williams *et al.*, 1974; Bastian, 1984). Bastian (1984) described cases of acute hepatic illness in four workers exposed from laying floors using an epoxy resin base containing MDA as a curing agent. The workers were exposed via fumes and dusts in the air as well from contact with powder to the hands and had worked with epoxy resins for periods ranging from one to 12 years. The level of exposure was not quantitated. The workers initially

reported to the hospital with symptoms of abdominal pain three days after the most recent exposure and all were discharged within four days. Two workers continued to show severe symptoms five days after the onset, with abdominal pain, jaundice, a tender liver, nausea, dyspnea, and muscular pain. Plasma bilirubin, alkaline phosphatase, and aspartate aminotransferase levels were elevated. Some symptoms did not subside until two months after the onset. One worker, after another exposure, experienced nausea, abdominal pain, and muscular pain. A second worker reported further symptoms of headache, tiredness, and decreased libido.

Symptoms in 6 of approximately 300 workers exposed to MDA by surface coating concrete walls with epoxy resins were reported (Williams *et al.*, 1974). Exposure probably occurred by inhalation, ingestion, and skin contact as a result of mixing powder containing MDA. Symptoms of clinical hepatitis in the 6 workers appeared two days to two weeks after beginning work, with five of the six having elevated bilirubin levels, and one liver biopsy showing bile stasis. All the workers recovered completely after an unspecified time.

Hepatitis among 13 men over the course of 6 years who were occupationally exposed to MDA in the blending of epoxy resins used in manufacturing insulating material was described (McGill and Motto, 1974). Among the 13 patients showing symptoms, all reported weakness, jaundice, and dark urine, 11 reported abdominal pain, nausea or vomiting, and anorexia; and over half reported fever, chills and/or headache. All the workers recovered within a 10 week period. After the first cases of hepatitis occurred, air sampling showed initial levels of MDA to be 0.1 ppm in the work area. After additional cases of hepatitis occurred, measures were taken to reduce worker exposure and air levels were reduced to as low as 0.0064 ppm. The authors concluded that percutaneous absorption was the likely major route of exposure in light of the facts that cases occurred in spite of measures taken to reduce air levels and there was evidence that significant hand contact with the compound occurred during the work day. Since the symptoms appeared within one to 18 days after “working intensively” with the compound and exposure routes were not clearly established, quantitation of exposure levels was considered difficult to establish.

The most well-known case of MDA toxicity to humans resulted from ingestion of bread made with flour contaminated with MDA during transport (Kopelman *et al.*, 1966a). Eighty-four persons showed symptoms of abdominal pain and some degree of jaundice. All patients had elevated serum alkaline phosphatase and glutamic oxaloacetic transaminase levels. Seventeen had serum bilirubin levels over 5 mg/100 ml. Liver biopsy was performed on 8 persons and evaluated in a separate study (Kopelman *et al.*, 1966b). The primary finding was an unusual lesion described during the early course of the disease as portal zone cholangitis and later as centrilobular cholestasis with necrosis. The initial study reported that all but 2 patients had complete recovery within several weeks, however, a two year follow-up study of 14 individuals showed that 10 still had symptoms of some severity 7-23 months after initial onset including food intolerance, gastrointestinal disturbances, fatigue, and visual disturbances (Kopelman, 1968).

Human effects other than hepatotoxicity have been described in the literature. Several cases of contact dermatitis and skin sensitization have been reported (LeVine, 1983; Van Joost *et al.*,

1987; de Pablo *et al.*, 1992; Bruynzeel and van der Wegen-Keijser, 1993). A case report of a man exposed to MDA with potassium carbonate and  $\gamma$ -butyrolactone by accidental ingestion has been described (Roy *et al.*, 1985). In addition to hepatitis and abnormal liver function which persisted over 18 months, the patient developed a progressively worsening retinopathy described as a “malfunction of the retinal pigment epithelium” accompanied by diminished visual acuity. The patient improved after approximately 3 months, but after examination at 18 months had not completely recovered.

Another report described the development of acute cardiomyopathy in addition to hepatitis in a worker exposed to a large quantity of MDA dust as the result of air filtration malfunction (Brooks *et al.*, 1979). The patient showed an abnormal ECG and an elevated cardiac LDH isoenzyme profile which returned to normal within one month of onset.

## **V. Effects of Animal Exposure**

The carcinogenicity of MDA was investigated in F344/N rats and B6C3F<sub>1</sub> mice (50/sex/dose group) administered in the drinking water at concentrations of 0, 150, and 300 ppm MDA (dihydrochloride) for 103 weeks (Lamb *et al.*, 1986). A 14-day range finding study was also conducted with 5 animal/sex/species/dose group, with exposure levels of 0, 200, 400, 800, 1600, and 3200 ppm MDA, and a 13-week subchronic study was conducted with 10 animals/sex/species/dose group, with exposure levels of 0, 25 (mice), 50, 100, 200, 400, and 800 (rats) ppm MDA. Using body weight and drinking water values from the study, low and high daily doses in the chronic study were calculated to be 9 and 16 mg/kg-day for male rats, 10 and 19 for female rats, 25 and 57 for male mice and 19 and 43 for female mice. In the chronic study, survival was reduced among male mice treated with 300 ppm MDA. Final mean body weights were reduced in the 300 ppm dose group of female rats (-9%), male mice (-13%), and female mice (-16%). Among rats, non-cancer effects included follicular cysts and follicular-cell hyperplasia of the thyroid (significantly increased incidence in high-dose females;  $p < 0.05$  by Fisher's exact test). In the liver, incidence of fatty and focal cellular change were elevated in low-dose male and female rats and also high dose male rats. Incidence of unspecified dilatation of the liver was also elevated in high-dose male rats. Increased incidence of kidney mineralization was found in male rats treated with 300 ppm MDA. Among mice, incidence of liver degeneration was elevated in males in both treatment groups and females in the high-dose group ( $p < 0.01$  by Fisher's exact test). Incidence of kidney nephropathy was increased in male and female mice in both treatment groups and mineralization of the renal papilla was increased in both sexes in the high-dose group ( $p < 0.01$ ). From the 13-week study, the authors noted thyroid and bile duct effects in rats at 800 ppm MDA and in mice at 400 ppm.

Albino and pigmented guinea pigs were exposed to aerosols of methylene dianiline in polyethylene glycol 200 (PEG) in nose-only exposure chambers (Leong *et al.*, 1987). Animals (8 of each strain) were exposed to a time-weighted average aerosol concentration of 0.44 g MDA/m<sup>3</sup> in air for 4 hours/day, 5 days/week for 2 weeks. Eight control animals were neither exposed to aerosol nor placed in the exposure chamber. Two weeks after the exposure period, animals were evaluated for dermal sensitization and irritation by challenge with 0.05 ml of 0, 2,

20 and 200 mg MDA/ml in PEG for up to 24 hours. No evidence of dermal irritation or sensitivity was found. Subsequently, the animals were also examined for pulmonary sensitization by challenge with aerosols containing 0.01 and 0.05 ml of 200 mg MDA/ml PEG. Lung insufflation pressures were measured as an indication of changes in lung distensibility. No evidence of pulmonary sensitization was found. After the pulmonary challenge, the animals were examined histopathologically, with emphasis on eye, lung, liver, and kidney toxicity. Ocular toxicity ranging from mild to more severe was observed in all MDA-treated animals, but in none of the control animals. Pigmented animals did not differ in sensitivity or effect compared to albino animals. Mild lesions were described as “retraction and thickening of the outer segments of the photoreceptor cells” while more severe effects included swelling “through the inner segments of the photoreceptor cells to the outer nuclear layer”. Some evidence of inflammatory cell infiltration was also noted and the pigmented epithelial layer was also degenerated. The authors conclude that the effects were attributable to MDA because no retinal lesions have been associated with exposure to the PEG vehicle. Furthermore, the inhalation exposures to MDA are the likely cause rather than the dermal and lung sensitization study exposures because these subsequent studies were conducted on control as well as treated animals. Pulmonary granulomas consisting of “an aggregate of macrophages surrounded by a thin mantle of lymphocytes” were found in 7 of the 16 MDA-exposed animals and one of the 8 control animals (level of significance was unstated). Treated and control animals had a high background incidence of pulmonary lesions including slight to mild bronchitis. No liver or kidney effects were detected in treated animals.

Nine purebred beagle dogs were treated orally (by capsule) with 70 mg “crude” (4 dogs) or “purified” (5 dogs) MDA in corn oil three days per week for a period ranging from approximately 3-7 years (Deichmann *et al.*, 1978). No concurrent controls were included since untreated animals were regularly maintained in the laboratory. After 2 years, cystoscopic examination was performed at 15-month intervals. After 4½ years, clinical chemistry tests were performed at 4 month intervals on 3 dogs from each group. Microscopic examination of urinary bladder, liver, heart, ovaries, uterus, and lymph nodes was performed on moribund animals or at the end of the experimental period (7 years, 2 months). Liver toxicity was noted in all the treated animals. Effects were described as fatty change, cell degeneration and necrosis, and lymphoid cell infiltration. One dog from each treatment group died from the toxic effect on the liver. The kidneys of four treated animals (two from each group) showed toxic effects including granuloma, glomerular nephritis, and congestion with cloudy swelling. Two dogs treated with “purified” and one dog treated with “crude” MDA showed toxicity to the spleen described as hemosiderosis and swelling with lymphocyte infiltration.

Wistar rats (5/sex/dose) were treated orally with 0, 0.0083 and 0.083 g MDA/kg body weight in propylene glycol daily for 12 weeks (Pludro *et al.*, 1969). Doses were 1% and 10% of the experimentally determined median lethal dose. No significant changes in body weight or hematological parameters were found, although serum albumin,  $\beta$ -globulin, and  $\gamma$ -globulin were elevated in animals in the 0.083 mg/kg dose group. The livers of all the animals in the high dose group showed signs of degeneration, with atrophy of the parenchyma and stromal hyperplasia in the portal areas. Also in this dose group, all animals showed hypertrophy of the lymphatic

nodules of the spleen. In the low dose group, one animal showed a liver lesion and one a lesion in the spleen.

Rats (8/sex) treated with MDA in 25% aqueous ethanol by stomach tube were examined (Schoental, 1968). Rats were given 20 mg doses a total of 2-5 times over several weeks up to 7½ months (frequency not specified). Animals showed necrosis of the liver and kidney and showed congestion and edema of the lungs.

Visual toxicity was reported in 15 cats treated perorally with 25-100 mg MDA/kg body weight in a 1% aqueous suspension (Schilling von Canstatt *et al.*, 1966). In four animals treated once with 100 mg/kg, no blindness was reported. In all the other treated animals (four with one dose of 100 mg/kg, two with one dose of 150 mg/kg, and two with three doses of 25 mg/kg and 3 doses of 50 mg/kg) blindness occurred within 8 days, three of which recovered sight within 4 days. Two other treated animals were examined microscopically, one treated with 25 and then 50 mg/kg and one treated once with 200 mg/kg. The first was examined after 7 days and showed signs of granular degeneration of the rods and cones with some proliferation of the pigmented epithelium. The second was examined after 4¼ years and showed atrophy of the retinal neuroepithelium. The authors note that no visual disturbances were found in other MDA treated experimental animals including dog, rabbit, guinea pig and rat.

## VI. Derivation of Chronic Reference Exposure Level (REL)

<i>Study</i>	Leong <i>et al.</i> , 1987
<i>Study population</i>	Guinea pigs
<i>Exposure method</i>	Discontinuous inhalation exposure (nose only) of aerosols
<i>Critical effects</i>	Degeneration of retinal epithelium
<i>LOAEL</i>	440 mg/m <sup>3</sup>
<i>NOAEL</i>	Not observed
<i>Exposure continuity</i>	4 hours/day, 5 days/week
<i>Exposure duration</i>	2 weeks
<i>Average experimental exposure</i>	52 mg/m <sup>3</sup> for LOAEL group
<i>LOAEL uncertainty factor</i>	10
<i>Subchronic factor</i>	10
<i>Interspecies uncertainty factor</i>	10
<i>Intraspecies uncertainty factor</i>	10
<i>Cumulative uncertainty factor</i>	3,000 (maximal uncertainty factor - due to lack of independence of 4 areas of uncertainty (USEPA, 1994))
<i>Inhalation reference exposure level</i>	0.02 mg/m <sup>3</sup> (20 µg/m <sup>3</sup> ; 0.002 ppm; 2 ppb)

Two specific types of toxicity have been associated with exposure to MDA: hepatotoxicity and ocular toxicity. Several studies have demonstrated hepatotoxicity in experimental animals, with the best study of long term toxicity of MDA reported by Lamb *et al.* (1986). In addition to

addressing the carcinogenicity of MDA, Lamb described non-cancer health effects which resulted from lifetime exposure to MDA at two concentrations in the drinking water of two species, rats and mice. The 150 ppm dose level was a LOAEL for fatty change and focal cellular change to the livers of male and female rats as well as for liver degeneration in male mice. The corresponding effects were also observed in high-dose male rats and male mice. Nephropathy was observed in mice of both sexes at the 150 and 300 ppm. There is abundant evidence from both human and animal studies that MDA is an hepatotoxic compound. Bastian (1984), Williams *et al.* (1974), and McGill and Motto (1974) reported hepatitis in people exposed by inhalation and dermal absorption routes. Kopelman *et al.* (1966a,b) demonstrated human hepatotoxicity from exposure by the oral route. Limited data detailing exposure levels associated with adverse health effects in humans precludes the development of a chronic REL from studies in humans.

The other toxic effect of potential concern from MDA exposure is ocular toxicity. Leong *et al.* (1987) reported damage to the retinas of guinea pigs exposed for 2 weeks to MDA aerosols ( $0.44 \text{ g/m}^3$  for 4 hr/day, 5 days/week for a average experimental exposure of  $52 \text{ } \mu\text{g/m}^3$ ) by inhalation. Schilling von Canstatt *et al.* (1966) also report blindness in cats treated orally with MDA. A single case of retinopathy and visual toxicity in humans was reported in a man who accidentally ingested MDA with potassium carbonate and  $\gamma$ -butyrolactone. The Leong *et al.* (1986) study was selected for the development of the chronic REL because, although conducted for a relatively short period of time, the study appears to address the most sensitive endpoint of toxicity by most appropriate route of exposure (inhalation). The suitable studies which establish the hepatotoxicity of MDA were conducted by the oral route of exposure.

The strengths of the inhalation REL include the availability of a controlled exposure inhalation study. Major areas of uncertainty are the lack of adequate human exposure data, the lack of chronic inhalation exposure studies, the lack of reproductive and developmental toxicity studies, the lack of observation of a NOAEL, and the use of animals under additional stress due to the restraint used to obtain nose-only exposure (control animals were not restrained). Potential liver toxicity has been included as a potential critical effect because of uncertainty regarding the relative potency of this compound in causing this effect in different species by different routes of exposure.

## VII. References

- ACGIH. 1992. American Conference of Governmental Industrial Hygienists, Inc. Documentation of the threshold limit values and biological exposure indices. Sixth edition. Cincinnati, OH.
- Bastian PG. 1984. Occupational hepatitis caused by methylenedianiline. *Med J Aust*, 141:533-535.
- Brooks LJ, Neale JM, and Pieroni DR. 1979. Acute myocardiopathy following tripathway exposure to methylenedianiline. *JAMA*, 242:1527-8.

Bruynzeel DP, and van der Wegen-Keijser MH. 1993. Contact dermatitis in a cast technician. *Contact Dermatitis*, 28:193-4.

de Pablo P, Ortiz J, Borrego L, Romero G, and Iglesias L. 1992. Allergic contact dermatitis from diaminodiphenylmethane in an ostomy bag. *Contact Dermatitis*, 27:260.

Deichmann WB, MacDonald WE, Coplan M, Woods F, and Blum E. 1978. Di-(4-aminophenyl)-methane (MDA): 4-7 year dog feeding study. *Toxicology*, 11:185-8.

HSDB. 1995. Hazardous Substances Data Bank. National Library of Medicine, Bethesda, Maryland (CD-ROM Version). Micromedex, Inc., Denver, Colorado (Edition expires 7/31/96).

Kopelman H. 1968. The Epping Jaundice after two years. *Postgrad Med J*, 44:78-81.

Kopelman H, Robertson MH, Sanders PG, and Ash I. 1966. The Epping Jaundice. *Br Med J*, 1:514-6.

Kopelman H, Scheuer PJ, and Williams R. 1966. The liver lesion of the Epping Jaundice. *Quebec Journal of Medicine*, 35:553-64.

Lamb JC, Huff JE, Haseman JK, Murthy ASK, and Lilja H. 1986. Carcinogenesis studies of 4,4'-methylenedianilinedihydrochloride given in drinking water to F344/N rats and B6C3F<sub>1</sub> mice. *J Toxicol Environ Health*, 18:325-37.

Leong BKJ, Lund JE, Groehn JA, Coombs JK, Sabatis CP, Weaver RJ, and Griffin RL. 1987. Retinopathy from inhaling 4,4'-methylenedianiline aerosols. *Fundam Appl Toxicol*, 9:645-58.

LeVine MJ. 1983. Occupational photosensitivity to diaminodiphenylmethane. *Contact Dermatitis*, 9:488-90.

McGill DB, and Motto JD. 1974. An industrial outbreak of toxic hepatitis due to methylenedianiline. *N Engl J Med*, 291:278-82.

Pludro G, Karlowski K, Mankowska M, Woggon H, and Uhde W-J. 1969. Toxicological and chemical studies of some epoxy resins and hardeners. I. Determination of acute and subacute toxicity of phthalic acid anhydride, 4,4'-diaminodiphenylmethane and of the epoxy resin: Epilox EG-34. *Acta Pol Pharm*, 26:352-7.

Roy CW, McSorley PD, and Syme IG. 1985. Methylene dianiline: a new toxic cause of visual failure with hepatitis. *Hum Toxicol*, 4:61-6.

Schilling von Canstatt B, Hofmann HT, Oettel H, and Zeller H. 1966. [Netzhautveränderungen der Katze bei der Vergiftung mit peroral oder perkutan verabreichten Chemikalien]. *Verhandlungen der Deutschen Gesellschaft für Pathologie*, 50:429-35.

Schoental R. 1968. Pathological lesions, including tumors, in rats after 4,4'-diaminodiphenylmethane and gamma-butyrolactone. *Isr J Med Sci*, 4:1146-58.

U.S.EPA. 1994. United States Environmental Protection Agency. Methods for derivation of inhalation reference concentrations and applications of inhalation dosimetry. Office of Research and Development, Washington DC.  
EPA/600/8-90/066F.

Van Joost T, Heule F, and de Boer J. 1987. Sensitization to methylenedianiline and para-structures. *Contact Dermatitis*, 16:246-8.

Williams SV, Bryan JA, Burk JR, and Wolf FS. 1974. Toxic hepatitis and methylenedianiline [letter]. *N Engl J Med*, 291:1256.

CHRONIC TOXICITY SUMMARY

# METHYLENE DIPHENYL ISOCYANATE POLYMER

(Diphenylmethane diisocyanate)

CAS Registry Number: 101-68-8

## I. Chronic Reference Exposure Level

<i>Inhalation reference exposure level</i>	<b>0.02 µg/m<sup>3</sup></b> (U.S. EPA RfC) This document summarizes the evaluation of non-cancer health effects by U.S. EPA for the RfC
<i>Critical effect(s)</i>	Hyperplasia of the olfactory epithelium in rats
<i>Hazard index target(s)</i>	Respiratory system

## II. Physical and Chemical Properties (HSDB, 1995)

<i>Description</i>	Light yellow solid
<i>Molecular formula</i>	C <sub>15</sub> H <sub>10</sub> N <sub>2</sub> O <sub>2</sub> (monomer)
<i>Molecular weight</i>	Variable (monomer = 250.27)
<i>Specific gravity</i>	1.197 @ 70°C (monomer)
<i>Boiling point</i>	196°C (monomer)
<i>Melting point</i>	37°C (monomer)
<i>Vapor pressure</i>	0.001 mm Hg @ 40°C (monomer)
<i>Solubility</i>	Soluble in acetone, benzene, kerosene, and nitrobenzene (monomer)
<i>Conversion factor</i>	Not applicable for polymer (for monomer 1 ppm = 10.2 mg/m <sup>3</sup> at 25°C)

## III. Major Uses or Sources

Methylene diphenyl isocyanate (MDI) is used for bonding rubber to nylon. MDI is also used in the manufacture of lacquer coatings and in the production of polyurethane resins and spandex fibers (HSDB, 1995).

#### IV. Effects of Human Exposure

A 5-year occupational study of 107 workers from a polyurethane plastic manufacturing plant examined pulmonary function, respiratory symptoms and smoking habits (Musk *et al.*, 1982). No significant changes in pulmonary function or respiratory symptoms were observed when controlled for smoking. Mean MDI concentrations measured ranged from 0.0003 to 0.0006 ppm.

Significant increased prevalences of asthma in female workers and chronic bronchitis in male and female workers were observed following occupational exposure to low levels of MDI (<0.02 ppm) (Pham *et al.*, 1988). Workers from two plants were grouped by job classification and evaluated in this study conducted in 1976; workers were grouped as unexposed (62 men, 21 women), indirectly exposed (61 men, 56 women), or directly exposed (91 men, 27 women). Further characterization of the exposure groups was not presented. Decrements in pulmonary function (measured by VC, FEV<sub>1</sub> and single-breath carbon monoxide diffusion tests) were observed in men in the direct and indirect exposure groups, decrements in men with a history of direct exposure to MDI were statistically significant. Workers were also grouped by duration of occupational exposure (<20 months, 20-60 months, >60 months). Workers with known (direct or indirect) occupational exposure to MDI for greater than 60 months exhibited statistically significant decrements in pulmonary function tests. The follow-up examination of this study describes data from male workers only. At the time of the 5-year follow-up, air levels had been reduced to below the maximum allowed air concentration of 0.005 ppm by a modification of the ventilation system. Statistically significant decrements in pulmonary function were observed again in workers with known direct occupational exposure to MDI. Workers who were exposed at the time of the 1976 study but whom had since been removed from exposure did not exhibit decrements in pulmonary function, leading the authors to conclude that the effects of low-level exposure to MDI are to some extent reversible. Flaws in study design, including lack of exposure characterization, attrition, and inclusion of asthmatics in cohorts, preclude a quantitative assessment of MDI exposure on lung function.

An epidemiologic study of foundry workers reported more respiratory symptoms, a significantly lower mean FEV<sub>1</sub> and maximum mid-expiratory flow at 25-75% in exposed workers compared to controls (Johnson *et al.*, 1985). However, MDI-exposed workers also had unquantified exposure to silica, metal dust, phenol formaldehyde, and a pyridine derivative precluding the evaluation of respiratory effects resulting from MDI exposure.

A worker with 5 years occupational exposure and suspected MDI hypersensitivity was exposed continuously in a controlled chamber to 5 ppb for 15 minutes then 10 ppb for 30 minutes and 20 ppb for 15 minutes (Marczynski *et al.*, 1992). The worker had not been exposed to MDI in the workplace for 5 days prior to the test challenge. Exposure to MDI resulted in an immediate moderate asthmatic reaction associated with significant hypoxemia.

## V. Effects of Animal Exposure

Rats were exposed to 0.2, 1.0, and 6.0 mg/m<sup>3</sup> aerosolized MDI polymer 6 hours per day, 5 days per week for 24 months (Reuzel *et al.*, 1994b). Statistically significant increases incidences of basal cell hyperplasia, olfactory epithelial degeneration, alveolar duct epithelialization, localized alveolar bronchiolization, and adenomas were observed male and female rats exposed to 6.0 mg/m<sup>3</sup> MDI. An accumulation of macrophages with yellow pigment was also noted in the lungs and mediastinal lymph nodes. Male rats exposed to this concentration also exhibited a statistically significant increase in the incidence of Bowman's gland hyperplasia. Male rats exposed to 1 mg/m<sup>3</sup> MDI also exhibited statistically significant increased incidences of basal cell hyperplasia and Bowman's gland hyperplasia. An accumulation of macrophages with yellow pigment was observed in the lungs of female rats and the lungs and mediastinal lymph nodes of male rats exposed to 1 mg/m<sup>3</sup>. No adverse effects were noted in rats exposed to 0.2 mg/m<sup>3</sup> MDI.

Guinea pigs were exposed to 2 ppm MDI 3 hours per day for 5 days (Aizicovici *et al.*, 1990). Qualitative immunostaining techniques indicated that MDI was localized in the respiratory tract. The spleen, lymph nodes, and thymus were had very little staining. However, another study exposed guinea pigs to 4 ppb radiolabelled toluene diisocyanate (TDI) for 1-hour and found measurable radioactivity in extrathoracic tissues and body fluids (Kennedy *et al.*, 1989). Therefore, there is a possibility that MDI may be transported to a sites other than the respiratory tract, such as the ovaries and testes, following inhalation exposure.

## VI. Derivation of U.S. EPA RfC

<i>Study</i>	Reuzel <i>et al.</i> , 1990; 1994 (evaluated by U.S. EPA, 1995)
<i>Study population</i>	Rats
<i>Exposure method</i>	Inhalation of polymeric aerosolized MDI (0.2, 1.0, and 6.0 mg/m <sup>3</sup> )
<i>Critical effects</i>	Hyperplasia of the olfactory epithelium
<i>LOAEL</i>	1 mg/m <sup>3</sup>
<i>NOAEL</i>	0.2 mg/m <sup>3</sup>
<i>Study continuity</i>	6 hours per day, 5 days per week
<i>Study duration</i>	24 months
<i>Average experimental exposure</i>	0.036 mg/m <sup>3</sup> for NOAEL group
<i>Human equivalent concentration</i>	0.005 mg/m <sup>3</sup> for NOAEL group (particle with extrathoracic respiratory effects, RDDR = 0.15, based on MMAD = 0.68 µm and sigma g = 2.93)
<i>LOAEL uncertainty factor</i>	1
<i>Subchronic uncertainty factor</i>	1
<i>Interspecies uncertainty factor (UF)</i>	3
<i>Intraspecies uncertainty factor (UF)</i>	10
<i>Modifying factor</i>	10 (database deficiencies)

<i>Cumulative uncertainty factor</i>	300
<i>Inhalation reference concentration</i>	0.02 µg/m <sup>3</sup>

Strengths of the RfC include the use of a well-conducted long-term inhalation study and the observation of a NOAEL. The major limitation of the MDI RfC is that it is based on data on exposures to MDI polymer. Monomers frequently are much more toxic than polymers, thus, while U.S. EPA reports the RfC as applying to MDI, OEHHHA considers the value is only predictive of adverse effects of polymeric MDI. Effects of monomeric MDI may occur at concentrations several orders of magnitude lower than observed in the reported study on MDI polymer.

## VII. References

Aizicovici S, Jin R, LaPietra D, Gottlieb F, and Karol MH. 1990. Use of immunohistochemistry to detect diphenyl methane 4,4-diisocyanate (MDI) in exposed guinea pigs. *The Toxicologist*. 10(1):286. Abstract No. 1144. Society of Toxicology. [cited in U.S. EPA, 1995].

CHRIS Hazardous Chemical Data. 1995. U.S. Department of Transportation, U.S. Coast Guard, Washington, D.C. (CD-ROM Version). Micromedex, Inc., Denver, Colorado (Edition expires 1/31/95).

HSDB. 1995. Hazardous Substance Data Bank. National Library of Medicine, Bethesda, Maryland (CD-ROM Version). Micromedex, Inc., Denver, Colorado (Edition expires 1/31/95).

Johnson A, Chan-Yeng M, MacLean L, *et al.* 1985. Respiratory abnormalities among workers in an iron and steel foundry. *Br. J. Ind. Med.* 42(2):94-100. [cited in U.S. EPA, 1995].

Kennedy AL, Stock MF, Alarie Y, Brown WE. 1989. Uptake and distribution of <sup>14</sup>C during and following inhalation exposure to radioactive toluene diisocyanate. *Tox. Appl. Pharmacol.* 100:280-292. [cited in U.S. EPA, 1995].

Marczynski B, Czuppon AB, Hoffarth HP, Marek W, and Baur X. 1992. DNA damage in human white blood cells after inhalative exposure to methylenediphenyl diisocyanate (MDI) - case report. *Tox. Letters* 60:131-138.

Musk AW, Peters JM, and Bernstein L. 1985. Absence of respiratory effects in subjects exposed to low concentrations of TDI and MDI: A reevaluation. *J. Occup. Med.* 27(12):917-920.

Musk AW, Peters JM, DiBerardinis L, and Murphy RLH. 1982. Absence of respiratory effects in subjects exposed to low concentrations of TDI and MDI. *J. Occup. Med.* 24(10):746-750.

Pham QT, Teculescu D, Meyer-Bisch C, and Mur JM. 1988. Effects of chronic exposure to diisocyanates. *Bull. Eur. Physiopathol. Respir.* 23:561-564.

Reuzel PGJ, Arts JHE., Lomax LG, Kuijpers MHM, Kuper CF, Gembardt C, Feron VJ, and Loser E. 1994. Chronic inhalation toxicity and carcinogenicity study of respirable polymeric methylene diphenyl diisocyanate (polymeric MDI) aerosol in rats. *Fund. Appl. Toxicol.* 22:195-210.

Reuzel PGJ, Arts JHE, Kuijpers MHM, and Kuper CF. 1990. Chronic toxicity/carcinogenicity inhalation study of polymeric methylene diphenyl diisocyanate aerosol in rats (Final report). Prepared by Civo Institute for the International Isocyanate Institute. Report No. V88.122. [cited in U.S. EPA, 1995].

Sulotto F, Romano C, Piolatto G, Coggiola M, Polizzi S, Ciacco C, and Berra A. 1990. Short-term respiratory changes in polyurethane foam workers exposed to low MDI concentration. *Int. Arch. Occup. Env. Health* 62:521-524.

U.S. EPA. 1995. U. S. Environmental Protection Agency. Integrated Risk Information System (IRIS). Office of Health and Environmental Assessment, Environmental Criteria Assessment Office, Cincinnati, OH. (CD-ROM Version).

Vogelmeier C, Baur X, and Fruhman G. 1991. Isocyanate-induced asthma: results of inhalation tests with TDI, MDI and metacholine. *Int. Arch. Occup. Env. Health* 63:9-13.